DIABETES SCREENING AND DIAGNOSIS IN AFRICAN AMERICANS: THE ROLE OF HEMOGLOBIN A1C

By Mary Elizabeth Lacy

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INTRODUCTION
Type 2 diabetes mellitus (T2DM) affects approximately 29 million adults in the United States population. African Americans adults, however, are disproportionately affected by T2DM, with 13% of all African American adults in the US affected compared to 7% of Whites.\textsuperscript{1} Further, African Americans are twice as likely as whites to have undiagnosed diabetes and are more likely than whites to experience a number of complications related to diabetes, including diabetic nephropathy, diabetic retinopathy and end-stage renal disease.\textsuperscript{2,3,4,5,6}

In most people, diabetes develops slowly and is characterized by an asymptomatic phase between onset of hyperglycemia and clinical diagnosis that is estimated to last for most patients between 4-7 years.\textsuperscript{7} Consequently, at any given time, somewhere around 30% of patients with diabetes are undiagnosed. Further, it’s estimated that, at the time of diagnosis, around 25% of patients have 1 or more diabetic complication.\textsuperscript{8} However, research has consistently shown that early intervention can reduce burden of disease, a fact which underscores the importance of effectively identifying those at increased risk so we can target them for intervention.\textsuperscript{9,10}

Historically, blood glucose measures were exclusively recommended to diagnose diabetes.\textsuperscript{11} Beginning in 2009, at the recommendation of an International Expert Committee, hemoglobin A1c (HbA1c) was added to diagnostic criteria for type 2 diabetes.\textsuperscript{12,13} HbA1c offers a number of advantages over traditional glucose-based measures: it doesn’t require fasting, it reflects average glucose control over the prior 2-3 months and it is a strong predictor of diabetic complications.\textsuperscript{14,15,16} However, it is not without controversy.
Data have consistently shown a racial difference in HbA1c levels. For a given glucose, HbA1c is 0.4%-0.6% higher in African Americans than in Whites.\textsuperscript{17,18,19} Yet the clinical implications of the difference remain unclear. One study reported that African Americans begin to have an increased risk of retinopathy at lower HbA1c values than Whites, while another study showed no racial difference in association of HbA1c with kidney disease or cardiovascular outcomes.\textsuperscript{20,21} Currently, there are no race-specific HbA1c thresholds for diagnosing or managing diabetes.

In order to target interventions to those at increased risk of diabetes, we need to be able to accurately identify those at increased risk by all measures, including the newly adopted HbA1c measures. Therefore, the objectives of this proposal were: 1) to examine if racial differences in HbA1c differences impact the ability to identify those at increased diabetes risk and 2) to examine underlying factors that are potentially contributing to this racial difference in HbA1c.
CHAPTER ONE

RACIAL DIFFERENCES IN THE PERFORMANCE OF EXISTING RISK PREDICTION MODELS FOR INCIDENT TYPE 2 DIABETES: THE CARDIA STUDY
INTRODUCTION

In 2010, the American Diabetes Association (ADA) modified the diagnostic guidelines for type 2 diabetes to include hemoglobin A1c (HbA1c). However, existing models for predicting diabetes risk were developed before the widespread adoption of HbA1c as a diagnostic test for diabetes. Thus, established diabetes risk prediction models do not include HbA1c. Additionally, most existing risk prediction models were developed in populations with few or no African Americans, despite the fact that Africa Americas are at increased risk of type 2 diabetes and vascular complications. Three of the more commonly used risk prediction models for incident type 2 diabetes were developed in the Atherosclerosis Risk in Communities (ARIC) Study (N=7,916; 85% White, 15% AA), the Framingham Offspring Study (N=3,140; 99% White), and the San Antonio Heart Study (N=3,004; 61% Mexican American, 39% White). An article comparing the validity of these three models in a multiethnic cohort reported good discrimination (AUC 0.78-0.81) for all three models, though model discrimination was lower in African Americans than Whites across all three models. Importantly, HbA1c was not included in any of these risk prediction models.

Findings from a number of studies have established that African Americans have higher HbA1c values than Whites, with estimates of the absolute HbA1c difference ranging from 0.40% to 0.65% after adjusting for glucose levels. Despite consistent evidence of higher HbA1c values in African Americans, the clinical significance of this difference is unclear. No racial differences were found for the association of HbA1c with incident coronary heart disease, stroke or chronic kidney disease in a prospective study of older African Africans and Whites; however, a cross-sectional study found that the
prevalence of retinopathy was elevated in African Americans versus Whites at the same HbA1c.\textsuperscript{14,15} These findings suggest that the benefit of HbA1c as a potential predictor of incident diabetes should be further explored in different racial groups. The objectives of this study were: 1) to examine the performance of an existing risk prediction model in a biracial cohort of African Americans and White adults from the Coronary Artery Risk Development Study in Young Adults (CARDIA) using ADA 2004 guidelines; 2) to examine model performance when diabetes status is ascertained using ADA 2010 guidelines; and 3) to examine change in model performance with the addition of baseline A1C as a predictor of diabetes risk.

With the recent changes to diagnostic guidelines for type 2 diabetes, we hypothesized that model discrimination would be lower using ADA 2010 diagnostic guidelines compared to ADA 2004 guidelines (the guidelines that were in use when existing models were developed). Additionally, we hypothesized that including baseline HbA1c as a predictor in the ADA 2010 model would improve prediction of diabetes in both Whites and African Americans. However, given the increased risk of diabetes in Africans Americans and HbA1c differences in African Americans compared to Whites, we hypothesized that model discrimination would be significantly lower in African Americans than Whites when HbA1c was incorporated into the prediction models.

METHODS

Study population
Details regarding the CARDIA study design have been published previously.\textsuperscript{16} In brief, CARDIA is an ongoing, multi-center longitudinal study of the determinants of cardiovascular disease in 5,115 adults aged 18-30 at the baseline assessment in 1985-1986. A stratified sample of 2,637 AA and 2,478 White men and women were recruited from Minneapolis, MN; Chicago, IL; Birmingham, AL; and Oakland, CA. Participants had follow-up examinations at 2, 5, 7, 10, 15, 20, and 25 years after enrollment. In CARDIA, fasting glucose was measured at baseline, and years 7, 10, 15, 20 and 25; 2-hour 75-gram oral glucose tolerance test was performed in years 10, 20 and 25; and A1C was measured at years 20 and 25. In order to construct a diabetes definition based on the current ADA diagnostic guidelines (which include HbA1c), analyses were restricted to follow-up examinations at which HbA1c was measured, i.e., years 20 and 25 of follow-up.

Therefore, the year 20 exam (2005-2006) was the baseline for the current study and incident diabetes was determined at the year 25 exam (2010-2011). A total of 3,549 participants completed the year 20 exam (74% of the surviving cohort). Participants with prevalent diabetes at the year 20 exam (n=332), and those who were missing diabetes status at year 20 (n=41) or year 25 (n=379), or who were missing covariate information (n=341) were excluded, resulting in 2,456 participants included in current study. Because fasting glucose levels were used as a covariate and in the outcome definition, analyses were restricted to participants who were fasting.

Data collection
CARDIA data was collected according to standardized protocols across the 4 study sites, as previously published in detail. All covariates were measured at the year 20 examination, except parental history of diabetes which was measured at the year 10 examination by self-report. Interviewers collected data on participants’ self-reported race, gender and date of birth at the baseline examination and verified these data at each subsequent examination. Self-reported medication use was ascertained by trained interviewers at the year 20 assessment. Height and weight were measured with participants wearing light clothing and no shoes. Body weight was measured to the nearest 0.2 kilogram using a calibrated balance-beam scale and height was measured with a vertical ruler to the nearest 0.5 cm; BMI was calculated as the ratio of weight in kilograms to height in meters squared. Waist circumference was measured to the nearest 0.5 centimeter at the minimum abdominal girth with participants standing upright; the average of two waist circumference measurements was used. Three seated blood pressure measurements were taken for each participant after a five-minute rest using an automated blood pressure monitor, with the average of the last two measurements used to determine systolic and diastolic blood pressure. Lipid assays were used to measure total, HDL, and LDL cholesterol, and triglycerides. At the year 20 and 25 follow-up, fasting and 2-hour post-load glucose were measured by the hexokinase ultraviolet method and HbA1c was assessed using a Tosoh G7 high performance liquid chromatography instrument. The coefficient of variation for all assays was <6%.

Risk prediction models
We calculated the 5-year predicted probability of developing type 2 diabetes for CARDIA participants based on the existing type 2 diabetes prediction model derived in the Atherosclerosis Risk in Communities Study (ARIC). The ARIC model was developed on 7,915 participants (85% non-Hispanic White, 15% African American) using approximately 9 years of follow-up starting in 1987-1989. The ARIC model includes the following predictors: age, race (African American vs. White), waist circumference, height, parent history of type 2 diabetes, systolic blood pressure, HDL cholesterol, triglycerides, and fasting glucose. We selected this model as our primary exposure as it is the only existing model that explicitly included an indicator variable for African American race. The ARIC model predicts incident diabetes over 9 years of follow-up. To account for the different length of follow-up available for CARDIA participants, we divided each CARDIA participant’s predicted probability by the number of years of follow-up used in the prior study (e.g. in ARIC, 9 years of follow-up) and multiplied this number by 5 to obtain the 5-year predicted probability of developing diabetes; this method assumes a constant risk of diabetes.

In sensitivity analyses, we examined the performance of two additional published type 2 diabetes prediction models from the Framingham Offspring Study and the San Antonio Heart Study. In contrast to the ARIC model, neither Framingham nor the San Antonio Heart Study had any African American participants. Thus, the diabetes prediction models from these two cohorts provide an interesting comparison. Model components and selected cohort characteristics are described in Table 2.

Assessment of Diabetes
We evaluated incident type 2 diabetes using two definitions: 1) using an outcome definition adapted from the ADA 2004 diagnostic guidelines which included reported use of anti-diabetic medication, fasting glucose ≥126 mg/dl, or 2-hour oral glucose tolerance test ≥200 mg/dL; and 2) using an outcome definition adapted from the ADA 2010 diagnostic guidelines which included reported use of anti-diabetic medication, or fasting glucose ≥126 mg/dl, 2-hour oral glucose tolerance test ≥200 mg/dL, or HbA1c ≥6.5%. Our definition of diabetes required only one elevated measure to define diabetes; confirmatory testing was not available.

In a sensitivity analysis, we examined an alternate definition of type 2 diabetes using an outcome definition adapted from the ADA 2010 diagnostic guidelines as indicated above with the addition of self-reported physician diagnosis of diabetes (i.e. reported use of anti-diabetic medication, or fasting glucose ≥126 mg/dl, 2-hour oral glucose tolerance test ≥200 mg/dL, or HbA1c ≥6.5% or self-reported physician diagnosis of diabetes).

Statistical analysis

Using the data from the CARDIA year 20 clinical assessment (the baseline for this study), we examined the distribution of demographics, anthropometrics, medical history and clinical covariates overall and by race. We also examined the 5-year incidence of type 2 diabetes in the overall population and stratified by race using both the ADA 2004 and ADA 2010 guidelines.

For each risk prediction model, we ran two sets of logistic regression models. The first set of models (Model 1) used CARDIA data and the regression coefficients
from the original published ARIC model to calculate the predicted probability of developing diabetes for each participant using ADA 2004 and ADA 2010 diagnostic guidelines. In the second set of models (Model 2), we re-fit the regression models using the same predictors from the original published ARIC model and estimated new regression coefficients using the CARDIA data. We also examined change in model performance after adding baseline HbA1c as a predictor of risk. All regression models were estimated for the overall cohort and in subgroups stratified by race. To determine if HbA1c results varied by other sociodemographic factors, we calculated the odds of developing type 2 diabetes including in subgroups defined by gender, age (<45 years old vs. ≥45 years old), and education level (did not complete high school vs. graduated high school) in addition to race (African American vs. White). Models were evaluated using three criteria to assess model fit: 1) model discrimination was evaluated using area under the receiver operating curve (AUC), a measure of how well the model ranked individuals who developed diabetes as at higher risk than those who did not;\textsuperscript{17,18} 2) model calibration which assesses how close the predicted risks are to the observed risks (summarized using the Hosmer-Lemeshow goodness of fit test);\textsuperscript{19} and 3) integrated discrimination improvement (IDI) after adding baseline HbA1c as a predictor.\textsuperscript{20,21,22} IDI is a measure of the separation in predicted probabilities for events and non-events across the ‘old’ (ARIC predictors only) and ‘new’ (ARIC predictors + HbA1c) model. Relative IDI compares the relative contribution of the new predictor (HbA1c) to the average contribution of the predictors from the original model. The original ARIC model has 9 predictors; assuming each variable contributes equally to the discrimination slope, the average contribution of each predictor is 11.1%. Estimates and
95% confidence intervals for absolute and relative IDI findings were estimated using 999 bootstrap replications with replacement.\textsuperscript{23} Analyses were conducted using SAS 9.3 statistical software (SAS Institute, Cary, NC).

RESULTS

Of the 2,456 participants included in analyses, 40.7% were African Americans and 59.3% were White. Whites tended to be older than African Americans, were more likely to be male, and had higher mean levels of triglycerides and fasting glucose. African Americans were more likely to have a parent with a history of type 2 diabetes, more likely to be prediabetic, and had significantly higher mean values of BMI, waist circumference, systolic blood pressure and HbA1c (Table 1).

The 5-year cumulative incidence of type 2 diabetes differed substantially when using the ADA 2004 diagnostic guidelines as compared to the ADA 2010 diagnostic guidelines. In the overall sample, the 5-year incidence of type 2 diabetes was 3.0% (n=74) under the ADA 2004 diagnostic guidelines versus 5.1% (n=124) using the ADA 2010 guidelines. African Americans were significantly more likely than Whites to develop diabetes using either guideline: under ADA 2004 diagnostic guidelines, 4.5% (n=45) of African Americans developed type 2 diabetes versus 2.0% (n=29) of Whites (p<0.0001); and under ADA 2010 guidelines, 7.6% (n=76) of African Americans developed type 2 diabetes versus 3.3% (n=48) of Whites (p<0.0001).

Components for the ARIC model are presented in Table 2. Using the previously published regression coefficients, the ARIC model yielded very high discrimination (AUC=0.846) for incident diabetes defined according to the ADA 2004 diagnostic
guidelines (Table 3, Model 1a). Model discrimination was slightly lower when incident diabetes was defined according to the ADA 2010 diagnostic guidelines (Model 1b; AUC=0.822 vs. 0.846, p=0.48 for difference between the two AUCs). Using the same set of predictors from the published ARIC model and re-estimating the regression equation in CARDIA, the prediction model achieved similar discrimination (AUC=0.841) for diabetes defined according to the ADA 2010 diagnostic guidelines (Model 2a). Adding baseline HbA1c as a covariate in the prediction model improved discrimination significantly (Model 2b; AUC=0.841 vs.0.863, p=0.03 for differences between the two AUCs). Hosmer-Lemeshow goodness of fit tests revealed no evidence of model misspecification for any of the models in the overall sample (all p-values >0.20).

Interaction terms examining the effect of HbA1c on diabetes risk in subgroups defined by gender, age, education level and race indicated that the effect of HbA1c did not differ in any of these subgroups except for race (p-values for interaction terms: gender* HbA1c, p-value=0.74; age* HbA1c, p-value=0.45; education* HbA1c, p-value=0.26; race* HbA1c, p-value=0.05).

Race-specific analyses revealed important differences in model discrimination. Across all models, model discrimination was higher among Whites than African Americans, though these differences were not all statistically significant. Using ADA 2004 diagnostic guidelines (Model 1a), model discrimination was higher in Whites than in African Americans though differences did not reach statistical significance (AUC in African Americans=0.802 vs. AUC in Whites=0.887, p=0.10). For all models that defined diabetes according to the ADA 2010 diagnostic guidelines (Models 1b, 2a, and 2b), model discrimination was significantly higher in Whites than in African Americans.
(Model 1b, AUC in African Americans=0.778 vs. AUC in Whites=0.860, p-value=0.04; Model 2a, AUC in African Americans=0.796 vs. AUC in Whites=0.875, p-value=0.04; Model 2b, AUC in African Americans=0.816 vs. AUC in Whites=0.902, p-value=0.008). Hosmer-Lemeshow goodness of fit tests revealed no evidence of model misspecification for any of the models in race-specific analyses (all p-values >0.20).

In the overall sample, the difference in mean predicted probabilities of type 2 diabetes between participants who developed diabetes and those who did not was 14.2% (Model 2a); this increased to 17.3% with the addition of predictor HbA1c (Model 2b) resulting in a statistically significant absolute IDI of 0.031 (95%CI=0.014, 0.047, p<0.0001) (Figure 1) and a relative IDI of 21.8% (95%CI=9.4%, 34.3%, p<0.0001). In African Americans, the absolute IDI was 0.029 (95%CI=0.006, 0.049, p<0.0001) and the relative IDI was 21.7% (95%CI=4.3%, 38.2%, p<0.0001). In Whites, the absolute IDI was 0.031 (95%CI=0.005, 0.055, p<0.0001) and the relative IDI was 22.8% (95%CI=4.3%, 43.4%, p<0.0001).

Findings from sensitivity analyses evaluating discrimination of the Framingham and San Antonio Heart Study models among CARDIA participants were similar to findings with the ARIC model (Supplemental Table S1). For each of these models, model discrimination was consistently higher in Whites than in African Americans. Results from a sensitivity analysis that examined an alternate outcome definition (which included self-reported physician diagnosis of diabetes) were similar to results from our main analysis (Supplemental Table S2).

CONCLUSIONS
In the current study, a previously published type 2 diabetes risk prediction model maintained high levels of discriminative validity when applied to a modern, biracial cohort of US adults. We found that model discrimination was high when using the ADA 2004 diagnostic guidelines among CARDIA participants. Defining incident diabetes using the ADA 2010 diagnostic guidelines, which include HbA1c, resulted in a decrease in model discrimination, which was reversed with the addition of baseline HbA1c as a predictor of type 2 diabetes risk. In the overall sample, there was no evidence of lack of model calibration. The addition of predictor HbA1c yielded a statistically significant increase in IDI and a relative IDI of 21.8%, a contribution which is well above the average contribution of the other 9 predictors in the ARIC model (11.1%). These findings suggest that including HbA1c in the prediction model results in significant improvement in model performance.

When racial subgroups in CARDIA were analyzed separately, model performance was better among Whites than African Americans. For all models that used ADA 2010 diagnostic guidelines, model discrimination was significantly higher in Whites than African Americans. The addition of baseline HbA1c improved model discrimination in Whites and African Americans to a similar degree, and IDI analyses suggested that HbA1c significantly improved model performance in both Whites and African Americans. Results from the current analysis confirm our hypothesis that when type 2 diabetes prediction models are updated to include HbA1c, a reflection of current clinical practice for the diagnosis of type 2 diabetes, there exists a racial divide in model performance. This difference in model performance may reflect racial differences in diagnostic practices among other factors. Therefore, we also examined other subgroups...
defined by gender, age, and education level to determine whether racial differences in model performance were being influenced by other key sociodemographic factors. These subgroup analyses indicated that the effect of HbA1c did not differ in any of these subgroups (p-values for interaction term >0.20 for all subgroups).

Potential limitations of this study included the availability of HbA1c measurements only at the year 20 and 25 follow-up. This short follow-up time (5 years) may have affected our study's power to detect significant differences in model performance, particularly among the racial subgroups. Also, our study defined diabetes based on modified diagnostic guidelines using reported use of antidiabetic medication, or a single measurement of fasting glucose, or 2-hour glucose or A1C HbA1c we did not have confirmatory testing available for fasting glucose. The diabetes definition used in our study differed slightly from the definition used in the ARIC study: both studies included fasting glucose and use of anti-diabetic medications; but our definition included 2-hour glucose where ARIC did not, and ARIC included self-report of diabetes diagnosis where our study did not. Because incident diabetes was assessed at the follow-up exam 5 years after baseline, our analyses assume a constant risk of diabetes over the study period and we were unable to assess whether the risk of diabetes varied at specific time points. Additionally, while it is not surprising that the inclusion of baseline HbA1c improved model performance, our findings highlight the extent to which addition of baseline HbA1c improves diabetes prediction in this biracial population and provides evidence of differential prediction by race in models with and without HbA1c. Lastly, the existing diabetes risk prediction models did not include lifestyle factors, such as diet and physical activity, so these variables were not evaluated in our study. Strengths of our
study included the use of a large, biracial cohort from four cities across the US with
detailed clinical and metabolic data available, including all of the recommended tests for
assessing diabetes status (i.e., fasting glucose, 2-hour glucose, and HbA1c).

Overall, an existing type 2 diabetes risk prediction model derived from the ARIC
Study maintained relatively strong discriminatory power in a biracial cohort comprised of
African Americans and Whites from four geographically diverse cities across the US.
Existing type 2 diabetes risk prediction models should be updated to incorporate ADA
2010 diagnostic criteria as these criteria reflect current clinical guidelines. Findings from
our analysis support the inclusion of HbA1c as a predictor of incident type 2 diabetes for
African Americans and Whites, yet suggest that, specifically in African Americans, there
is a need for closer examination of the optimal prediction of diabetes risk. Further
investigation in additional cohorts that include racial and ethnic minorities is warranted.
Table 1. Characteristics of CARDIA study participants at Year 20 (2005-2006)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
<th>African American</th>
<th>White</th>
<th>p-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=2,456</td>
<td>n=999</td>
<td>n=1,457</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.29 ± 3.56</td>
<td>44.59 ± 3.81</td>
<td>45.78 ± 3.30</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Male (%)</td>
<td>43.04</td>
<td>38.24</td>
<td>46.33</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.80 ± 6.39</td>
<td>30.55 ± 6.81</td>
<td>27.60 ± 5.78</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.42 ± 14.35</td>
<td>92.37 ± 14.18</td>
<td>89.08 ± 14.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Parental history of diabetes (%)</td>
<td>17.79</td>
<td>22.82</td>
<td>14.34</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>114.58 ± 14.07</td>
<td>118.62 ± 15.43</td>
<td>111.81 ± 12.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>54.87 ± 16.65</td>
<td>55.24 ± 16.16</td>
<td>54.62 ± 16.98</td>
<td>0.37</td>
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<tr>
<td>Triglycerides (mg/dL)</td>
<td>106.16 ± 73.15</td>
<td>94.32 ± 68.28</td>
<td>114.27 ± 75.27</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>94.33 ± 9.35</td>
<td>93.72 ± 10.37</td>
<td>94.74 ± 8.56</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.34 ± 0.37</td>
<td>5.46 ± 0.41</td>
<td>5.26 ± 0.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HbA1c (mmol/mol)</td>
<td>35 ± 4.0</td>
<td>36 ± 4.5</td>
<td>34 ± 3.4</td>
<td>&lt;0.0001</td>
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<td>Prediabetes (%)†</td>
<td>39.05</td>
<td>46.55</td>
<td>33.91</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 1 Footnote: *Unless otherwise noted, results presented as mean ± SD; †Comparison by χ² for categorical variables and unpaired t tests for continuous variables; ‡ Prediabetes defined as: fasting plasma glucose 100-125 mg/dL, A1C 5.7-6.4% or oral glucose tolerance test 140-199 mg/dL
Figure 1. Change in predicted probability of developing diabetes under ADA 2010 guidelines in CARDIA participants with the addition of baseline HbA1c to predict diabetes risk.
Table 2. Summary of select existing type 2 diabetes prediction models and characteristics of original cohort

<table>
<thead>
<tr>
<th>Study/version</th>
<th>N</th>
<th>Average follow-up</th>
<th>Baseline for model development</th>
<th>Population sample</th>
<th>Variables</th>
<th>Outcome Ascertainment</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC:</td>
<td>7,915</td>
<td>9 years</td>
<td>1987-1989</td>
<td>85% White, 15% AA</td>
<td>Age, race, waist circumference, height, parental history of diabetes, HDL, triglycerides, fasting plasma glucose, systolic blood pressure</td>
<td>Elevated 2-hour glucose, elevated fasting glucose, diabetes medications or report of a clinical diagnosis during follow-up</td>
</tr>
<tr>
<td>Clinical model plus fasting glucose and lipids</td>
<td></td>
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</tr>
<tr>
<td>Framingham:</td>
<td>3,140</td>
<td>7 years</td>
<td>Mid 1990's</td>
<td>99% White; 0% AA</td>
<td>Age, sex, BMI, waist circumference, parent history of diabetes, HDL, triglycerides, fasting plasma glucose, systolic blood pressure</td>
<td>Diabetic medications or elevated fasting plasma glucose</td>
</tr>
<tr>
<td>Multivariate prediction with continuous variables</td>
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<tr>
<td>San Antonio Heart Study: Clinical model with no 2-hour glucose</td>
<td>3,004</td>
<td>7.5 years</td>
<td>1979-1982 and 1984-1988</td>
<td>61% Mexican American, 39% White; 0% AA</td>
<td>Age, sex, Mexican American ethnicity, BMI, family history of diabetes, HDL, fasting plasma glucose, systolic blood pressure</td>
<td>Diabetic medications, elevated fasting plasma glucose, elevated 2-hour glucose, or self-report of physician diagnosis</td>
</tr>
<tr>
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</tbody>
</table>

Table 2 Footnote: Prediction models used to calculate probability (diabetes) = exp(X) / (1 + exp(X)); ARIC Study logistic regression model: X = -9.9808 + 0.0173*age in years + 0.4433*African American race + 0.4981*1 if parent history of diabetes is present + 0.0880*FPG in mg/dL + 0.0111*SBP in mm Hg + 0.0273*waist circumference in cm – 0.0326*height in cm – 0.0122*HDL cholesterol in mg/dL + 0.00271*triglycerides in mg/dL; Framingham Study logistic regression model: X = -18.607 - 0.0101*age in years -0.4308*sex (1 if male, 0 if female) + 0.4383*1 if parent history of diabetes if present + 0.03922*BMI + 0.001*SBP in mm Hg - 0.0488*HDL in mg/dL + 0.0488*waist circumference in cm + 0.1398*FPG in mg/dL; San Antonio Heart Study logistic regression model: X = -13.415 + 0.028*age in years + 0.661*sex (1 if female, 0 if male) + 0.412*1 if Mexican American (all 0 for this study) + 0.079*FPG in mg/dL + 0.018*SBP in mm Hg – 0.039*HDL in mg/dL + 0.070*BMI + 0.481*1 if family history of diabetes is present.
Table 3. Discrimination of 5-year incident diabetes using the ARIC risk prediction model: the CARDIA study (2005 – 2011)

| Model | Overall AUC (95% CI) | African Americans AUC (95% CI) | Whites AUC (95% CI) | p-value*
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>Model 1: Previously published regression coefficients</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1a: ADA 2004 diagnostic guidelines</td>
<td>0.846 (0.794, 0.898)</td>
<td>0.802 (0.721, 0.884)</td>
<td>0.887 (0.827, 0.947)</td>
<td>0.10</td>
</tr>
<tr>
<td>Model 1b: ADA 2010 diagnostic guidelines</td>
<td>0.822 (0.782, 0.862)</td>
<td>0.778 (0.716, 0.840)</td>
<td>0.860 (0.814, 0.906)</td>
<td>0.04</td>
</tr>
<tr>
<td>p-value†</td>
<td>0.48</td>
<td>0.64</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>Model 2: Regression equations re-estimated in CARDIA using ADA 2010 diagnostic guidelines</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 2a: Original predictors</td>
<td>0.841 (0.806, 0.876)</td>
<td>0.796 (0.737, 0.854)</td>
<td>0.875 (0.830, 0.920)</td>
<td>0.04</td>
</tr>
<tr>
<td>Model 2b: Original predictors + A1C</td>
<td>0.863 (0.832 0.894)</td>
<td>0.816 (0.763, 0.869)</td>
<td>0.902 (0.867, 0.936)</td>
<td>0.008</td>
</tr>
<tr>
<td>p-value‡</td>
<td>0.03</td>
<td>0.14</td>
<td>0.08</td>
<td></td>
</tr>
</tbody>
</table>

Table 3 Footnote: *p-value for unpaired ROC comparison of models estimated in African Americans versus Whites; †p-value for unpaired ROC comparison of models estimated within Overall cohort, African Americans and Whites comparing change in model discrimination when diagnostic guidelines are updated from ADA 2004 to ADA 2010 diagnostic guidelines; ‡p-value for paired ROC comparison of models estimated within Overall cohort, African Americans and Whites comparing change in model discrimination when diagnostic guidelines are updated to include baseline A1C as a predictor
CHAPTER TWO

ASSOCIATION OF SICKLE CELL TRAIT WITH HEMOGLOBIN A1C IN AFRICAN AMERICANS
INTRODUCTION

Hemoglobin A1c (HbA1c) is a practical measure of average glucose levels during the preceding 2 to 3 months.\(^1\)\(^-\)\(^4\) In 2009, after review of available evidence, an International Expert Committee recommended the use of HbA1c to diagnose diabetes.\(^5\) Extensive work has been done by the NGSP (formerly, the National Glycohemoglobin Standardization Program) to standardize methods for measuring HbA1c and to identify methods that provide accurate HbA1c measurement even in the presence of hemoglobin variants.\(^6\)

Sickle cell trait (SCT) is the most common hemoglobin variant in the United States, with 8% to 10% of black people affected by SCT compared with less than 1% of white people.\(^7\)\(^,\)\(^8\) Red blood cells of individuals with normal hemoglobin contain approximately 97% HbA, whereas red blood cells of individuals with SCT contain approximately 60% to 70% HbA and 30% to 40% HbS.\(^9\) Although data are limited, it is hypothesized that the presence of HbS results in a shorter lifespan for red blood cells.\(^9\)\(^-\)\(^12\) This would result in less available time for hemoglobin glycation, which in turn may influence the interpretation of HbA1c in relationship to the glucose values they intend to represent. Correct interpretation of HbA1c values in individuals with SCT is important because it directly affects efforts that use HbA1c for screening, diagnosis, and monitoring of diabetes and prediabetes. Accordingly, the objectives of this study were to (1) examine the association between HbA1c and SCT while controlling for other measures of glucose (fasting and 2-hour glucose) levels, (2) compare the prevalence of prediabetes and diabetes by SCT status, and (3) determine if SCT modifies the discriminative ability of HbA1c to identify individuals with prediabetes or diabetes.
METHODS

Study Population

This retrospective study pooled data from participants who self-identified as African American from 2 established community-based cohorts, the Coronary Artery Risk Development in Young Adults (CARDIA) study and the Jackson Heart Study (JHS), to examine the association of SCT with HbA1c, controlling for fasting glucose or 2-hour glucose levels. Details regarding the design of each study have been published.\textsuperscript{13,14} In brief, the CARDIA study enrolled, from March 25, 1985 to June 7, 1986, a stratified sample of 2637 black people and 2478 white people ($n = 5115$) from Minneapolis, Minnesota; Chicago, Illinois; Birmingham, Alabama; and Oakland, California. After their initial visit, participants were followed up approximately 2, 5, 7, 10, 15, 20, and 25 years later (data collection, 1985-present). The Jackson Heart Study, a single-site study in Jackson, Mississippi, enrolled 5301 African Americans during the years 2000 through 2004. Participants returned for 2 follow-up examinations approximately 5 and 10 years after their initial visit (data collection, 2000-2013). The data used for these analyses were from years 20 and 25 from the CARDIA cohort and from baseline and years 5 and 10 from the JHS cohort. All participants included in analyses provided written informed consent for genetic studies. Institutional review board approval was obtained separately from each participating institution.

Covariate Assessment
Details regarding data collection procedures for CARDIA\textsuperscript{15,16} and JHS\textsuperscript{17} have been published. Data on participants’ sex, self-reported race, date of birth, diet, physical activity, smoking status, medical history, and medication use were collected at each visit by trained interviewers. Physical activity was classified as poor (0), intermediate (>0-<150), or ideal (≥150) using minutes per week of moderate to vigorous physical activity. Diet was classified as poor (0-1), intermediate (2-3), or ideal (4-5) using the number of components achieved from the following list: 4.5 or more cups of fruits and vegetables daily; 198 g or more of fish weekly; less than 1500 mg of sodium daily; less than 450 calories each week of sugar-sweetened beverages; and 3 or more servings daily of whole grains. Smoking was classified as poor (current smoker), intermediate (quit <12 months ago) or ideal (never smoked or quit ≥12 months ago). Height and weight were measured by certified study personnel and used to calculate body mass index (BMI), weight in kilograms divided by height in meters squared. Estimated glomerular filtration rate (eGFR) was calculated at years 20 and 25 in CARDIA and at baseline and year 10 in JHS using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.\textsuperscript{18} Serum ferritin was measured at year 20 in CARDIA and at baseline in JHS. Previous diabetes diagnosis was defined as self-report of a physician’s diagnosis. Current use of diabetes medications was defined as use of diabetes medications in the 2 weeks prior to the examination.

SCT Status

In CARDIA, SCT status was determined with available DNA samples from the 10-year follow-up and afterwards using single-gene, single-nucleotide polymorphism
(SNP) genotyping with TaqMan SNP Genotyping Assays (Life Technologies). In JHS, genotype data for rs334 encoding the sickle hemoglobin mutation (HBB p.Glu7Val) was obtained through whole-exome sequencing using data from baseline. Sickle cell trait was defined as the presence of 1 abnormal allele for HbS.

Plasma Glucose Measures

Plasma glucose was measured using the hexokinase method at all visits in both studies except at baseline in JHS, at which time the glucose oxidase method was used. Previous research has shown these 2 methods of glucose measurement to be highly correlated.\textsuperscript{19,20} Additionally, 2-hour glucose levels were measured in CARDIA and obtained during a standard oral glucose tolerance test, using 75 g of glucose solution. The coefficient of variation for glucose measures ranged from 1.6\% to 3.8.

HbA1c Measures

Two NGSP-certified assays were used to measure HbA1c, both using high-performance liquid chromatography. In JHS, a Tosoh 2.2 was used at baseline and a Tosoh G7 (variant mode) was used at the 5- and 10-year follow-ups. In CARDIA, a Tosoh G7 (variant mode) was used at the 20- and 25-year follow-up. According to the NGSP, neither Tosoh 2.2 nor Tosoh G7 (variant mode) has experienced clinically significant interference in those with SCT.\textsuperscript{6} The coefficient of variation for HbA1c assays ranged from 1.2\% to 1.9\%.

Main Outcome Measure
The main outcome measure, HbA1c, was assessed at multiple time points and pooled cross-sectionally to examine the association between HbA1c, modeled as a continuous variable, and SCT, adjusting for fasting or 2-hour glucose measures. Prespecified secondary outcomes include prediabetes, diabetes, and combined prediabetes or diabetes, which were defined using the following measures based on cut-points established by the American Diabetes Association\textsuperscript{21}:(1) fasting glucose levels (prediabetes, 100-<126 mg/dL; diabetes, ≥126 mg/dL; and combined prediabetes or diabetes, ≥100 mg/dL), 2-hour glucose levels (prediabetes, 140-<200 mg/dL; diabetes, ≥200 mg/dL; and combined pre-diabetes or diabetes, ≥140 mg/dL), and HbA1c levels (prediabetes, 5.7%-<6.5%; diabetes, ≥6.5%; combined prediabetes or diabetes, ≥5.7%). (To convert glucose from mg/dL to mmol/L, multiply by 0.0555.)

Statistical Methods

Baseline characteristics of participants with and without SCT were compared using χ2 tests and analysis of variance for discrete and continuous variables, respectively. All visits with concurrent measurement of HbA1c and fasting glucose or HbA1c and 2-hour glucose levels were included in the analyses. Mean HbA1c values were calculated by SCT status across a range of glucose categories in 10-mg/dL increments for fasting glucose (<80-≥150 mg/dL) and in 20-mg/dL increments for 2-hour glucose levels (<80-≥200 mg/dL).

Generalized estimating equations (GEE) using random effects at the participant level and an exchangeable correlation matrix to account for correlation among repeated measures were used to assess the association of SCT with HbA1c controlling for fasting
glucose levels. A multistep approach was used to examine the robustness of findings to model specification. First, unadjusted GEE models were fit using HbA1c as the outcome, SCT as the exposure, and glucose as the primary covariate. Next, GEE analyses were adjusted for the following potential confounders that were identified a priori based on the literature: age, sex, BMI, ferritin levels, eGFR, physician-diagnosed diabetes, use of diabetes medications, and study cohort. In addition, multiplicative SCT × glucose interaction was tested in unadjusted and adjusted models. Analyses were repeated using 2-hour glucose measures in lieu of fasting glucose measures as the main covariate. Model fit was compared using quasi-likelihood under the independence-model criterion. Prespecified subgroup analyses were conducted among participants not taking diabetes medications and in groups of participants stratified by cohort. Interaction by cohort and HbA1c assay on the association between SCT and HbA1c was tested.

Next, among a subset of participants with no previous diabetes or current use of diabetes medications, GEE analyses with a Poisson distribution and an identity link function were used to estimate the unadjusted prevalence of prediabetes and diabetes by SCT status.

In the same subset of participants, GEE analyses with a Poisson distribution and a log link function (sometimes called a modified Poisson model) were used to generate predictive probabilities that were then used in logistic regression models to compare the discriminative ability of HbA1c levels to identify the combined presence of prediabetes or diabetes. A combined outcome of prediabetes or diabetes was used due to consideration of sample size. Area under the receiver operating characteristic (AUROC)
curves were calculated for HbA1c levels in those with and without SCT. Unpaired comparisons of the AUROC curves were conducted to assess the discriminatory power of HbA1c by SCT status. Complete case analysis was performed for adjusted analyses because data were more than 98% complete, with the exception of ferritin (7% missing). A 2-sided P value of ≤ .05 was used for level of significance. Analyses were performed using SAS statistical software version 9.4 (SAS Institute Inc).

RESULTS

Baseline Characteristics

A total of 7938 participants were enrolled at baseline, of whom 2637 were in CARDIA and 5301 in JHS. One thousand sixty-five CARDIA and 2253 JHS participants were excluded. Of those excluded in CARDIA, 550 had no available SCT data; 3 had HbSS; 2, HbCC; 41, HbAC; and 469, no concurrent measurements of HbA1c or fasting or 2-hour glucose measurements. Of those excluded in JHS, 2079 had no available SCT data; 2, HbSS; 80, HbAC; and 92, no concurrent measurements of HbA1c and fasting or 2-hour glucose. A total of 4620 participants (58.2%) were included in these analyses—1572 in CARDIA and 3048 in JHS.

The 367 participants (7.9%) with SCT were older (53.9 vs 52.2 years, P = .007) and had lower eGFR (92.5 vs 97.5 mL/min/1.73 m2, P < .001), and lower HbA1c values (5.7% vs 5.9%, P = .001; Table 1) than did participants without SCT. Additionally, participants with SCT were more likely to report previously diagnosed diabetes (17.2%
vs 14.7%, P=.20) and current use of diabetes medications (15.0% vs 12.5%, P=.19),
although these findings did not reach statistical significance.

Association of HbA1c with Glucose Measures

For all 4620 participants, each visit with concurrent HbA1c and fasting glucose or
HbA1c and 2-hour glucose measures was included, resulting in 9062 concurrent
measures of HbA1c and fasting glucose (2583 from CARDIA; 6479 from JHS) from
4620 unique participants and 2001 concurrent measures of HbA1c and 2-hour glucose
measures (2001 from CARDIA) from 1323 unique participants. The majority of
participants (74.6%) had HbA1c and glucose measured at least twice. Using all
available observations, the mean HbA1c was 5.7% in those with SCT vs 6.0% in those
without SCT, despite similar mean fasting (103.0 vs 102.9 mg/dL; P=.88) and 2-hour
glucose values (118.5 vs 113.0 mg/dL; P=.19) for those with SCT vs those without SCT,
respectively. Across all categories of fasting and 2-hour glucose measures, mean
HbA1c values were lower in those with vs without SCT (Table 2).

Unadjusted GEE analyses revealed that, at the same fasting glucose
concentration, HbA1c values were statistically significantly lower in those with SCT
(mean HbA1c, 5.72%) vs those without SCT (mean HbA1c, 6.01%; mean HbA1c
difference, −0.29%; 95% CI, −0.35% to −0.23%; P < .001). In adjusted analyses, HbA1c
values remained significantly lower in those with SCT (mean HbA1c difference, −0.32%;
95% CI, −0.38% to −0.26%; P < .001). The difference in HbA1c levels by SCT status
was greater at higher concentrations of fasting glucose (P = .02 for interaction in
unadjusted analyses, Figure 1A; P = .01 in adjusted analyses, Figure 1B). There was no
evidence of interaction by HbA1c assay (P = .43 for SCT × fasting glucose × HbA1c assay) or by study cohort (P = .63 for SCT × fasting glucose × study cohort).

Results for 2-hour glucose measures revealed similar HbA1c differences by SCT status. For a given 2-hour glucose level, HbA1c values were statistically significantly lower in those with SCT (mean HbA1c, 5.35%) vs in those without SCT (mean HbA1c, 5.65%), for a mean HbA1c difference of −0.30% (95% CI, −0.39% to −0.21%; P < .001). HemoglobinA1c levels remained significantly lower in those with SCT in adjusted analyses (mean difference in HbA1c, −0.38%; 95% CI, −0.49% to −0.28%; P < .001). The difference in HbA1c levels by SCT status was greater at higher 2-hour glucose concentrations (P = .03 for interaction in unadjusted analyses, Figure 1C; P = .03 in adjusted analyses, Figure 1D).

Prevalence of Prediabetes and Diabetes

Among a subset of participants with no prior diagnosis of diabetes or current use of diabetes medications (7449 observations for fasting glucose and HbA1c; 1869 observations for 2-hour glucose and HbA1c), the prevalence of prediabetes and diabetes was not significantly different among participants with vs without SCT when defined using fasting glucose (28.6% with vs 25.0% without SCT for prediabetes; P = .12; 2.5% with vs 3.6% without SCT for diabetes; P = .25) or 2-hour glucose values (15.9% with vs 12.9% without SCT for prediabetes, P = .45; and 3.6% with vs 3.3% without SCT for diabetes; P > .89; Figure 2). In contrast, the prevalence of prediabetes and diabetes was statistically significantly lower among participants with SCT when
defined using HbA1c values (29.2% with vs 48.6% without SCT for prediabetes and 3.8% with vs 7.3% without SCT for diabetes; P < .001 for all comparisons, Figure 2).

Discriminative Ability of HbA1c to Identify Prediabetes or Diabetes

In the same subset of participants without diabetes or diabetes medication use, the discriminative ability of HbA1c to identify the presence of prediabetes or diabetes was statistically significantly lower among participants with SCT (AUROC, 0.70; 95% CI, 0.65-0.74) vs without SCT (AUROC, 0.77; 95% CI, 0.75-0.78; absolute difference, 0.07; 95% CI, 0.02-0.12) when using fasting glucose–defined prediabetes or diabetes (P < .01, Figure 3A). The same held true among participants with SCT (AUROC, 0.60; 95% CI, 0.47-0.72) vs those without SCT (AUROC, 0.74; 95% CI, 0.71-0.78; absolute difference, 0.15; 95% CI, 0.02-0.28) when using 2-hour glucose-defined prediabetes or diabetes measures (P = .02, Figure 3B).

DISCUSSION

In this retrospective cohort study of African Americans participating in 2 large US cohorts, we demonstrated that, at the same fasting or 2-hour glucose concentration, HbA1c is statistically significantly lower among participants with vs without SCT. Moreover, differences in HbA1c concentration by SCT status were greater at higher glucose concentrations. These differences are based on an HbA1c method reported to have no clinically significant interference in individuals with SCT.6,31 Our findings stand in contrast to 2 previous studies. Bleyer and colleagues32 investigated the HbA1c-glucose association in 85 African American inpatients, the majority of whom had
diabetes and among whom there was an unusually high prevalence of SCT: 109 (28%) had SCT. Despite higher baseline HbA1c values, the authors did not find that SCT significantly altered the relationship between HbA1c and serum glucose. Sumner and colleagues \(^3\) examined the sensitivity of HbA1c to detect impaired glucose tolerance in a cohort of 216 African immigrants without diabetes, 46 (21%) of whom had either HbC trait or SCT. They found no significant difference in the sensitivity of HbA1c by variant hemoglobin status in the detection of prediabetes; however, this study combined participants with SCT and HbC trait, which may have affected findings. Additionally, both of these studies are potentially limited by their small sample size.

We consider 2 ways in which SCT could modify the ability of HbA1c to accurately reflect past glycemia. First, the life-span of the red blood cells in persons with SCT may be shortened compared with those with normal hemoglobin, resulting in less time available for glycation. However, the evidence to support this hypothesis remains limited and conflicting.\(^9\)-\(^12\) Second, the presence of HbS can result in assay interference with common HbA1c measurement techniques. Current testing on HbA1c laboratory methods uses a relative bias of plus or minus 7.0% to classify clinically significant interference from hemoglobin variants.\(^6\) Although the assays used in this study report no clinically significant interference in individuals with SCT, the possibility of minor interference that could potentially explain our findings cannot be ruled out.

Irrespective of the mechanism, our results suggest that currently accepted clinical measures of HbA1c do not reflect recent past glycemia in the same way in African Americans with and without SCT, as evidenced by significantly lower HbA1c values at the same glucose concentration in those with vs without SCT. These results
could have clinically significant implications. As a screening tool, an HbA1c value that systematically underestimates long-term glucose levels may result in a missed opportunity for intervention. In the present study, using standard clinical HbA1c criteria to identify prediabetes and diabetes resulted in identifying 40% fewer cases of prediabetes and 48% fewer cases of diabetes among participants with SCT compared with those without SCT, while glucose-based methods resulted in a similar prevalence regardless of SCT status (Figure 2). The discriminative ability of HbA1c concentration to identify individuals with prediabetes or diabetes was significantly lower in those with vs those without SCT (Figure 3). These findings raise the possibility of benefit from incorporating information on hemoglobin variants into clinical guidelines for interpreting HbA1c values for screening and diagnosis of prediabetes and diabetes. Because black people typically have a higher prevalence of diabetes and experience a number of diabetic complications at higher rates than white people, the cost of inaccurately assessing risk and treatment response is high.34-36

In our study, African Americans with and without SCT had relatively high HbA1c levels with mean values greater than 5%. This is possibly due to the higher values often found in black people than in white people.37,38 Future studies that include biracial populations should further investigate HbA1c differences by race in relation to hemoglobinopathies. We also noted that individuals with SCT had a lower eGFR as was observed previously by Naik and colleagues.39 Future studies should focus on whether a possible delay in the diagnosis and treatment of prediabetes and diabetes in those with SCT could explain their lower kidney function.
Strengths of our study include the availability of detailed information on demographics, medical history, and clinical measures as well as the reproducibility of findings across 2 different versions of the Tosoh HbA1c assay (2.2 and G7) and across 2 different measures of glucose concentration (fasting and 2-hour glucose).

This study has a number of limitations. Despite pooling data from 2 large cohorts, a relatively small number of participants had SCT (367 participants). Although, to our knowledge, this is the largest study to date examining the association between HbA1c, SCT, and other glucose measures, further studies including biracial populations and other hemoglobin variants are needed to confirm and complement our findings. Second, validation of these findings with other HbA1c assays would help explain the potential mechanisms of the disparate HbA1c-glycemia association between those with and without SCT. Third, even though multiple measures of glucose were available and findings were consistent with fasting and 2-hour glucose measures, these measures cannot fully represent average glycemia over the prior 2 to 3 months, which might lower the precision of our estimates. However, our findings were robust in analyses adjusted for potential confounding variables and in various sensitivity analyses suggesting that the likelihood of such bias is low. Fourth, a large number of participants were excluded from analyses due to missing data on SCT status, HbA1c measures, or glucose measures; however, participants excluded were comparable with those in the analytic sample with the exception of a lower BMI at baseline among those excluded. In addition, no clinical outcomes were assessed in this study. Whether the findings in this study have clinical implications is unclear.
CONCLUSIONS

Among African Americans from 2 large, well-established cohorts, participants with SCT had lower levels of HbA1c at any given concentration of fasting or 2-hour glucose compared with participants without SCT. These findings suggest that HbA1c may systematically underestimate past glycemia in black patients with SCT and may require further evaluation.
Table 1. Baseline characteristics of study participants by sickle cell trait status

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall</th>
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<th>JHS only</th>
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<td></td>
<td>N=4,620</td>
<td>n=4,253</td>
<td>n=1,467</td>
<td>n=2,786</td>
</tr>
<tr>
<td></td>
<td>n=367</td>
<td>p-value</td>
<td>n=105</td>
<td>n=262</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>1,785 (38.6)</td>
<td>1,634 (38.4)</td>
<td>1,036 (37.2)</td>
<td>1,036 (37.2)</td>
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<tr>
<td>Age, mean (SD), y</td>
<td>52.3 (11.8)</td>
<td>51.3 (11.8)</td>
<td>45.7 (4.3)</td>
<td>45.7 (4.3)</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>31.8 (7.4)</td>
<td>31.8 (7.5)</td>
<td>31.5 (7.7)</td>
<td>31.5 (7.7)</td>
</tr>
<tr>
<td>Hemoglobin, mean (SD), mg/dL</td>
<td>13.0 (1.5) (^{b})</td>
<td>13.1 (1.5) (^{b})</td>
<td>124.0 (148.0) (^{c})</td>
<td>169.7 (179.1) (^{c})</td>
</tr>
<tr>
<td>Ferritin, mean (SD), ng/mL</td>
<td>155.9 (169.7) (^{c})</td>
<td>156.3 (171.8) (^{c})</td>
<td>107.2 (98.7) (^{c})</td>
<td>165.0 (152.6) (^{c})</td>
</tr>
<tr>
<td>eGFR, mean (SD), ml/min/1.73m(^{2})</td>
<td>97.1 (21.5)</td>
<td>97.5 (21.3)</td>
<td>103.0 (19.7)</td>
<td>94.6 (21.5)</td>
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<td>Fasting glucose, mean (SD), (mg/dL)</td>
<td>101.6 (33.2)</td>
<td>101.5 (33.2)</td>
<td>101.1 (30.2)</td>
<td>101.7 (34.6)</td>
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<td>HbA1c, mean (SD), %</td>
<td>5.9 (1.2)</td>
<td>5.9 (1.2)</td>
<td>5.8 (1.0)</td>
<td>6.0 (1.3)</td>
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<tr>
<td>2-hour glucose, mean (SD), (mg/dL)</td>
<td>113.9 (44.5) (^{d})</td>
<td>113.8 (44.9) (^{d})</td>
<td>113.8 (44.9)</td>
<td>-- (^{d})</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>685 (14.9)</td>
<td>622 (14.7)</td>
<td>213 (14.5)</td>
<td>409 (14.7)</td>
</tr>
<tr>
<td>Diabetes medication, n (%)</td>
<td>577 (12.75)</td>
<td>523 (12.53)</td>
<td>116 (7.9)</td>
<td>407 (15.0)</td>
</tr>
</tbody>
</table>

Table 1 Footnotes:

Abbreviations: CARDIA, Coronary Artery Risk Development in Young Adults Study; JHS, Jackson Heart Study; SCT, sickle cell trait; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); eGFR, estimated glomerular filtration rate (calculated using the CKD-EPI equation); HbA1c hemoglobin A1c.

SI conversion factor: to convert glucose from mg/dL to mmol/L, multiply by 0.0555.

\(^{a}\) Baseline for this study was the first visit at which a participant had A1C and fasting or 2-hour glucose measured concurrently. For CARDIA participants, this was either year 20 or year 25 follow-up exam. For JHS participants, this was either exam 1, 2, or 3.

\(^{b}\) Hemoglobin was only measured in 2,976 participants all of whom are from JHS (ie, 64% of the total analytic sample).

\(^{c}\) Ferritin was only measured in 4,278 participants (ie, 93% of the total analytic sample).

\(^{d}\) 2-hour glucose was only measured in 1,222 participants all of whom are from CARDIA and are not on diabetes medications (ie, 26% of the total analytic sample).

\(^{e}\) Diagnosed diabetes is defined as self-reported use of diabetes medications or self-reported physician diagnosis.

\(^{f}\) Diabetes medications is defined as use of a medication for the treatment of diabetes taken in the 2 weeks prior to the study exam.
<table>
<thead>
<tr>
<th>Fasting glucose, mg/dL</th>
<th>Non-trait</th>
<th></th>
<th></th>
<th>SCT</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total # of observations</td>
<td># of unique participants</td>
<td>HbA1c %, mean (SD)</td>
<td>Total # of observations</td>
<td># of unique participants</td>
<td>HbA1c %, mean (SD)</td>
</tr>
<tr>
<td>&lt;80</td>
<td>482</td>
<td>429</td>
<td>5.6 (0.9)</td>
<td>46</td>
<td>40</td>
<td>5.4 (0.6)</td>
</tr>
<tr>
<td>80-99</td>
<td>2,207</td>
<td>1,683</td>
<td>5.5 (0.5)</td>
<td>167</td>
<td>130</td>
<td>5.3 (0.5)</td>
</tr>
<tr>
<td>90-99</td>
<td>2,712</td>
<td>2,073</td>
<td>5.7 (0.5)</td>
<td>243</td>
<td>183</td>
<td>5.5 (0.5)</td>
</tr>
<tr>
<td>100-109</td>
<td>1,394</td>
<td>1,154</td>
<td>6.0 (0.6)</td>
<td>120</td>
<td>105</td>
<td>5.6 (0.6)</td>
</tr>
<tr>
<td>110-119</td>
<td>513</td>
<td>460</td>
<td>6.3 (0.7)</td>
<td>58</td>
<td>53</td>
<td>5.9 (0.6)</td>
</tr>
<tr>
<td>120-129</td>
<td>268</td>
<td>241</td>
<td>6.8 (0.9)</td>
<td>22</td>
<td>20</td>
<td>6.4 (0.8)</td>
</tr>
<tr>
<td>130-139</td>
<td>165</td>
<td>154</td>
<td>7.0 (0.9)</td>
<td>9</td>
<td>9</td>
<td>6.6 (0.5)</td>
</tr>
<tr>
<td>140-149</td>
<td>128</td>
<td>124</td>
<td>7.4 (1.2)</td>
<td>11</td>
<td>11</td>
<td>6.9 (0.9)</td>
</tr>
<tr>
<td>≥150</td>
<td>473</td>
<td>379</td>
<td>9.3 (2.1)</td>
<td>44</td>
<td>33</td>
<td>8.5 (1.9)</td>
</tr>
<tr>
<td>Overall</td>
<td>8,342</td>
<td>4,253</td>
<td>6.0 (1.2)</td>
<td>720</td>
<td>367</td>
<td>5.7 (1.0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Two-hour glucose, mg/dL</th>
<th>Total # of observations</th>
<th># of unique participants</th>
<th>HbA1c %, mean (SD)</th>
<th>Total # of observations</th>
<th># of unique participants</th>
<th>HbA1c %, mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;80</td>
<td>269</td>
<td>230</td>
<td>5.4 (0.4)</td>
<td>14</td>
<td>14</td>
<td>5.2 (0.3)</td>
</tr>
<tr>
<td>80-99</td>
<td>507</td>
<td>440</td>
<td>5.5 (0.4)</td>
<td>33</td>
<td>30</td>
<td>5.2 (0.3)</td>
</tr>
<tr>
<td>100-119</td>
<td>529</td>
<td>465</td>
<td>5.6 (0.4)</td>
<td>29</td>
<td>26</td>
<td>5.4 (0.3)</td>
</tr>
<tr>
<td>120-139</td>
<td>268</td>
<td>244</td>
<td>5.7 (0.5)</td>
<td>26</td>
<td>23</td>
<td>5.5 (0.2)</td>
</tr>
<tr>
<td>140-159</td>
<td>130</td>
<td>123</td>
<td>5.8 (0.5)</td>
<td>8</td>
<td>8</td>
<td>5.3 (0.3)</td>
</tr>
<tr>
<td>160-179</td>
<td>69</td>
<td>66</td>
<td>5.9 (0.5)</td>
<td>9</td>
<td>8</td>
<td>5.6 (0.4)</td>
</tr>
<tr>
<td>180-199</td>
<td>37</td>
<td>35</td>
<td>6.2 (0.5)</td>
<td>3</td>
<td>3</td>
<td>5.6 (0.5)</td>
</tr>
<tr>
<td>≥200</td>
<td>65</td>
<td>59</td>
<td>7.6 (2.4)</td>
<td>5</td>
<td>5</td>
<td>6.5 (1.5)</td>
</tr>
<tr>
<td>Overall</td>
<td>1,874</td>
<td>1,239</td>
<td>5.7 (0.7)</td>
<td>127</td>
<td>84</td>
<td>5.4 (0.5)</td>
</tr>
</tbody>
</table>

Table 2 Footnotes:
Abbreviations: HbA1c, hemoglobin A1c; SCT, sickle cell trait.
SI conversion factor: to convert glucose from mg/dL to mmol/L, multiply by 0.0555.
Figure 1. Scatterplot of Observed Data Model of Hemoglobin A1c vs Fasting and 2-Hour Glucose Measures in Participants with or without Sickle Cell Trait

A.

B.

C.
Figure 1 Legend:
Scatterplot of observed data points alongside unadjusted and adjusted regression lines examining the association between sickle cell trait (SCT) and hemoglobin A1c (HbA1c), controlling for fasting or 2-hour glucose values was obtained using generalized estimating equations (GEE) with an exchangeable correlation matrix to account for correlation of repeated measures. All continuous covariates are centered at the population mean. The white dots represent observed data points for participants without SCT and the black dots represent observed data points for participants with SCT. The solid lines represent the regression line for those for who did not have SCT and the dashed lines for those who had SCT. BMI indicates body mass index; CARDIA, Coronary Artery Risk Development in Young Adults study. SI conversion factor: to convert glucose from mg/dL to mmol/L, multiply by 0.0555.

A, included 9062 observations, 720 from participants with SCT and 8342 from participants without SCT. The regression equation: predicted HbA1c = 6.01 + (−0.28 × SCT) + (0.03 × fasting glucose) + (−0.004 × SCT fasting glucose).
B. Included 8460 observations, 683 from participants with SCT and 7777 from participants without SCT. The regression equation: predicted HbA1c = \(5.93 + (-0.32 \times \text{SCT}) + (0.03 \times \text{fasting glucose}) + (-0.005 \times \text{SCT} \times \text{fasting glucose}) + (0.04 \times 1 \text{ if male}) + (0.008 \times \text{age}) + (0.01 \times \text{BMI}) + (-0.0004 \times \text{ferritin}) + (0.001 \times \text{estimated glomerular filtration rate}) + (-0.08 \times 1 \text{ if a CARDIA participant}) + (0.46 \times 1 \text{ if currently using diabetes medications}) + (0.14 \times 1 \text{ if previous diabetes diagnosis})\).

C. Included 2001 observations, 127 from participants with SCT and 1874 from participants without SCT. Regression equation: predicted HbA1c = \(5.65 + (-0.28 \times \text{SCT}) + (0.01 \times 2\text{-hour glucose}) + (-0.004 \times \text{SCT} \times 2\text{-hour glucose})\).

D. Included 1712 observations, 109 from participants with SCT and 1606 from participants without SCT. Regression equation: predicted HbA1c = \(5.66 + (-0.36 \times \text{SCT}) + (0.01 \times 2\text{-hour glucose}) \times (-0.004 \times \text{SCT} \times 2\text{-hour glucose}) + (0.24 \times 1 \text{ if male}) + (0.02 \times \text{age}) + (0.006 \times \text{BMI}) + (-0.0006 \times \text{ferritin}) + (0.0003 \times \text{eGFR}) + (0.07 \times 1 \text{ if previous diabetes diagnosis})\).
Figure 2. Prevalence of Prediabetes and Diabetes by Sickle Cell Trait Status among Participants Not Taking Diabetes Medications and with no Prior Diagnosis of Diabetes

Figure 2 Legend:
Fasting glucose and hemoglobin A1c analyses included 7499 total observations (6877 observations from participants without sickle cell trait [SCT] and 572 from participants with it). Analyses for 2-hour glucose concentrations were only available from CARDIA participants and included 1869 total observations (1752 observations from participants without SCT and 117 from participants with SCT). For the definition of prediabetes and diabetes by glucose measures, see the Methods section. The prevalence of prediabetes and diabetes by fasting glucose and 2-hour glucose concentration was similar in those with and without SCT (P > .10 for all comparisons). However, the prevalence of prediabetes and diabetes as defined by hemoglobin A1c was significantly higher among participants with vs without SCT (P < .001 for both). Error bars indicate 95% CIs. Non-trait: Light gray, SCT: Dark gray.
Figure 3. Comparison of the Diagnostic Sensitivity of Hemoglobin A1c to Identify Combined Prediabetes or Diabetes by Sickle Cell Trait Status

A.

B.
Figure 3 Legend:

A, Non-trait: Solid line, SCT: Dashed line; For fasting glucose of 100 mg/dL or higher, the area under the receiver operating characteristic (AUROC) of hemoglobin A1c (HbA1c) was 0.77 (95% CI, 0.75-0.78) among those without sickle cell trait (SCT) and 0.70 (95% CI, 0.65-0.74) among those with SCT. An unpaired comparison of the AUROC curves indicated that the diagnostic ability of HbA1c to identify fasting glucose–defined prediabetes or diabetes was significantly lower among those with SCT than among those without it (P = .007).

B, Non-trait: Solid line, SCT: Dashed-line; For 2-hour glucose levels of 140 mg/dL or higher, the AUROC of HbA1c was 0.74 (95% CI, 0.71-0.78) among those without SCT and 0.60 (95% CI, 0.47-0.72) among those with SCT. An unpaired comparison of the AUROC curves indicated that the diagnostic ability of HbA1c to identify 2-hour glucose-defined prediabetes or diabetes was significantly lower in those with SCT than in those without it (P = .03). To convert glucose from mg/dL to mmol/L, multiply by 0.0555.
CHAPTER THREE

INCIDENCE OF DIABETES AND PREDIABETES BY SICKLE CELL TRAIT STATUS IN AFRICAN AMERICANS
INTRODUCTION

African Americans are at increased risk of type 2 diabetes and many diabetic complications.1,2,3 Since 2010, hemoglobin A1c (HbA1c) has been used to screen for and diagnose diabetes.4 HbA1c offers a number of advantages over traditional glucose-based measures, however, the use of HbA1c remains controversial in certain populations for example in individuals with certain hemoglobinopathies.5,6 Sickle cell trait (SCT) is the most common hemoglobinopathy in the United States with a prevalence of 8-10% in African Americans as compared to <1% of Whites.7 The use of HbA1c to screen for, diagnose and monitor glycemic control in those with SCT is recommended as long as an assay is used that does not experience clinically significant interference from SCT.8 However, a recent study reported significantly lower HbA1c values for any given fasting or 2-hour glucose value and a significantly lower prevalence of diabetes and prediabetes in African Americans with SCT than in those without despite using an assay that is approved for use in those with SCT.9 The impact of SCT on the ability of HbA1c to identify incident cases of diabetes and prediabetes, however, remains unknown. To address this knowledge gap, here, we examine the incidence of diabetes and prediabetes using a variety of glucose and HbA1c-based definitions among 2,971 African Americans from two established community-based cohort studies.

RESEARCH DESIGN AND METHODS

This retrospective cohort study pooled data on African Americans from two community-based cohorts, the Coronary Artery Risk Development in Young Adults Study (CARDIA) and the Jackson Heart Study (JHS). Details regarding the design of
each study have been previously published.\textsuperscript{10,11} Data used in this analysis were measured in CARDIA at Year 20, 25 and 30 follow-up exams (2005-2016) and in JHS at baseline, Year 5 and 10 follow-up exams (2000-2013). SCT was defined as the presence of one abnormal allele for hemoglobin S and was determined using single gene, single nucleotide polymorphism in CARDIA and whole exome sequencing in JHS. Plasma glucose was measured using the hexokinase method at all visits in both studies except at baseline in JHS, where the glucose oxidase method was used. In CARDIA, 2-hour glucose was obtained during a standard oral glucose tolerance test using 75g of glucose solution. Two assays (Tosoh 2.2 and Tosoh G7 variant mode) were used to measure HbA1c. According to the NGSP, neither Tosoh 2.2 nor Tosoh G7 (variant mode) experiences clinically significant interference in those with SCT.\textsuperscript{12} The main outcome measures include prediabetes and diabetes using: fasting glucose (prediabetes: 100-<126 mg/dL; diabetes: $\geq$126 mg/dL), 2-hour glucose (prediabetes: 140-<200 mg/dL; diabetes: $\geq$200 mg/dL), and HbA1c (prediabetes: 5.7-<6.5%; diabetes $\geq$6.5%).

Participants with prevalent diabetes at baseline were excluded from analyses. Baseline characteristics of participants with and without SCT were compared using $\chi^2$ tests and ANOVA for discrete and continuous variables, respectively. We calculated the crude 10-year cumulative incidence of prediabetes and diabetes in the overall sample as well as by SCT status and compared incidence by SCT status using $\chi^2$ tests. A two-sided p-value of $\leq$0.05 was used for level of significance. Analyses were performed using SAS 9.4 (Cary, NC).
RESULTS

A total of 2,971 African Americans were included in analyses, of whom 246 had SCT (8.28%). Baseline characteristics for participants with and without SCT were similar (Table 1), except participants with SCT were older (mean (SD) = 54.33 (12.59) years vs 51.55 (11.93) years) and had lower HbA1c values (mean (SD) = 5.36% (0.46%) vs 5.51% (0.44%)).

Over 10 years of follow-up, of the 2,971 participants included in analyses, a total of 149 participants (5.02%) developed incident diabetes using fasting glucose (≥126 mg/dL) and 310 participants (10.43%) developed incident diabetes using HbA1c (≥6.5%). The incidence of fasting glucose-defined diabetes did not differ by SCT status (5.69% vs 4.95% in those with vs without SCT; p=0.61). When using HbA1c-defined diabetes, however, the incidence was statistically significantly lower in those with SCT (6.50% vs 10.79% in those with vs without SCT; p=0.04). In a subset of participants from CARDIA with 2-hour glucose measured (n=798), the incidence of 2-hour glucose-defined diabetes (≥200 mg/dL) was statistically significantly higher in those with SCT than in those without SCT (9.43% vs 3.22% in those with vs without SCT; p=0.04).

Among 1,746 without prevalent prediabetes at baseline, a total of 388 participants (22.22%) developed incident prediabetes using fasting glucose (≥100 mg/dL) and 763 participants (43.70%) developed incident prediabetes using HbA1c (≥5.7%). Incidence did not differ by SCT status when using fasting glucose-defined prediabetes (26.97% vs 21.77% in those with vs without SCT; p=0.14) but was, again, statistically significantly lower in those with SCT when using HbA1c-defined prediabetes (24.34% vs 45.55% in those with vs without SCT; p<0.0001). Among CARDIA
participants with 2-hour glucose measured, the incidence of 2-hour glucose-defined prediabetes was higher in those with SCT though findings did not reach statistical significance (20.00% vs 9.82% in those with vs without SCT; p=0.06).

CONCLUSIONS

In this analysis of data collected on 2,971 African Americans from two large, community-based cohort studies, we found that the 10-year incidence of diabetes and prediabetes differed significantly by SCT status when using HbA1c- and 2-hour glucose-based definitions. Interestingly, these observations were in opposing directions.

When using HbA1c-based definitions, over 10 years of follow-up, there were 40% fewer cases of incident diabetes and 47% fewer cases on incident prediabetes in those with SCT. In contrast, when using 2-hour glucose-based definitions, there were nearly 3 times more cases of incident diabetes and twice as many cases of incident prediabetes in those with SCT compared to those without. Fasting glucose-based definitions revealed comparable incidence of both diabetes and prediabetes in the two groups.

Previous research found that HbA1c may be underestimating glycemia in African Americans with SCT, yet it was unknown whether or not these findings would have clinical implications for the diagnosis of new cases of diabetes. Our results suggest that, in this population, the underestimation of glycemia in those with SCT is of a great enough magnitude to impact diagnosis.

However, findings must be interpreted with caution. Although the HbA1c assays used in this study are not reported to have clinically significant interference from SCT, it
is possible that findings are attributable to the particular HbA1c assay that was used. Further, the classifications used to define incident diabetes and prediabetes in this study are based on one elevated glucose or HbA1c measure as opposed to the two measures recommended in clinical practice to confirm the diagnosis.

Additionally, this is the first study to report an elevated incidence of diabetes and prediabetes in those with SCT using 2-hour glucose measures. Though fasting glucose-based definitions did not reach statistical significance, the incidence of both diabetes and prediabetes was slightly higher in the SCT compared the non-SCT group. Taken as a whole, these findings may suggest an actual increase in diabetes risk among African Americans with SCT though the underlying mechanism remains unclear. If confirmed, these findings would also add to the growing evidence that SCT may not merely be a benign carrier state, but, rather, may have potentially important clinical consequences.13,14

In conclusion, we found that, among African Americans from 2 well-characterized cohorts, using HbA1c to identify incident cases of prediabetes and diabetes resulted in a significantly lower incidence of both conditions among those with SCT while using 2-hour glucose resulted in a higher incidence. HbA1c-findings are likely attributable to underestimation of glycemia in those with SCT. The mechanism explaining increased incidence among those with SCT when using glucose-based measures remains unclear and deserves further investigation.
Table 1. Baseline characteristics of study participants by sickle cell trait status

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Overall (N=2971)</th>
<th>No SCT (n=2725)</th>
<th>SCT (n=246)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, mean (SD), y</td>
<td>51.79 (12.01)</td>
<td>51.55 (11.93)</td>
<td>54.33 (12.59)</td>
<td>0.001</td>
</tr>
<tr>
<td>Men, No. (%)</td>
<td>1074 (37.83)</td>
<td>975 (37.47)</td>
<td>99 (41.77)</td>
<td>0.19</td>
</tr>
<tr>
<td>BMI, mean (SD)</td>
<td>31.29 (7.22)</td>
<td>31.28 (7.20)</td>
<td>31.41 (7.53)</td>
<td>0.79</td>
</tr>
<tr>
<td>Waist circumference, mean (SD), cm</td>
<td>97.38 (15.71)</td>
<td>97.32 (15.64)</td>
<td>98.09 (16.50)</td>
<td>0.46</td>
</tr>
<tr>
<td>Parental history of diabetes, No. (%)</td>
<td>931 (31.98)</td>
<td>857 (32.11)</td>
<td>74 (30.58)</td>
<td>0.63</td>
</tr>
<tr>
<td>SBP, mean (SD), mmHg</td>
<td>123.94 (16.63)</td>
<td>123.93 (16.66)</td>
<td>123.97 (16.33)</td>
<td>0.98</td>
</tr>
<tr>
<td>HDL cholesterol, mean (SD), mg/dL</td>
<td>53.18 (15.41)</td>
<td>53.30 (15.33)</td>
<td>51.76 (16.32)</td>
<td>0.19</td>
</tr>
<tr>
<td>Triglycerides, mean (SD), mg/dL</td>
<td>95.74 (55.11)</td>
<td>95.82 (55.79)</td>
<td>94.79 (46.33)</td>
<td>0.81</td>
</tr>
<tr>
<td>Homa-IR</td>
<td>3.61 (2.39)</td>
<td>3.59 (2.36)</td>
<td>3.81 (2.73)</td>
<td>0.24</td>
</tr>
<tr>
<td>hsCRP, mean (SD), (ug/mL)</td>
<td>1.66 (3.75)</td>
<td>1.68 (3.83)</td>
<td>1.49 (2.65)</td>
<td>0.49</td>
</tr>
<tr>
<td>Fructosamine (umol/L)*</td>
<td>226.62 (20.60)</td>
<td>226.36 (20.64)</td>
<td>230.61 (19.70)</td>
<td>0.19</td>
</tr>
<tr>
<td>Glycated albumin (%)*</td>
<td>13.00 (1.30)</td>
<td>12.99 (1.28)</td>
<td>13.24 (1.60)</td>
<td>0.15</td>
</tr>
<tr>
<td>2-hour glucose, mean (SD), mg/dL*</td>
<td>106.89 (30.53)</td>
<td>106.70 (30.45)</td>
<td>109.45 (31.72)</td>
<td>0.51</td>
</tr>
<tr>
<td>Fasting glucose, mean, (SD), mg/dL</td>
<td>91.30 (9.90)</td>
<td>91.31 (9.78)</td>
<td>91.24 (11.24)</td>
<td>0.93</td>
</tr>
</tbody>
</table>

HbA1C, mean (SD), % 5.50 (0.44) 5.51 (0.44) 5.36 (0.46) <0.0001

Abbreviations: SCT (Sickle Cell Trait), BMI (Body Mass Index), HDL (High Density Lipoprotein), SBP (Systolic Blood Pressure), HbA1C (Hemoglobin A1c)

*Fructosamine (n=833), glycated albumin (n=833) and 2-hour glucose (n=840) were only measured in CARDIA
Figure 1. Ten-year cumulative incidence of diabetes and prediabetes by SCT status

**Figure 1 Legend:** Diabetes analyses using fasting glucose- and HbA1c-based definitions included 2,971 total participants (2,727 without SCT and 246 with SCT). Diabetes analyses using 2-hour glucose-based definitions included 798 total participants (745 without SCT and 53 with SCT) all of whom were from CARDIA. Prediabetes analyses using fasting glucose- and HbA1c-based definitions included 1,746 total participants (1,594 without SCT and 152 with SCT). Prediabetes analyses using 2-hour glucose-based definitions included 539 total participants (499 without SCT and 40 with SCT) all of whom were from CARDIA.
CONCLUSION
The addition of hbA1c to the diagnostic guidelines for diabetes in 2010 represented a major shift in clinical practice. Since this change in guidelines, hbA1c has been widely adopted in practice largely due to the decreased burden it places on patients as compared to fasting or 2-hour glucose measures and the ability of hbA1c to reflect longer-term glucose control. However, there remains considerable controversy surrounding the use of hbA1c in particular patient populations.

Our first paper, "Racial differences in the performance of existing risk prediction models for incident type 2 diabetes: The CARDIA study," revealed significant racial differences in the performance of diabetes prediction models to identify those at increased risk of developing diabetes. Importantly, racial differences were more pronounced in models that incorporated hbA1c suggesting the need to further evaluate the potential causes and implications of racial differences in hbA1c.

In our second paper, "Association of sickle cell trait with hemoglobin A1c in African Americans," we focused on one particular condition that may be differentially impacting hbA1c in African Americans, SCT. We found that SCT interfered with accurate assessment of hbA1c in African Americans which was resulting in an underestimation of glycemia in those with SCT.

In our final paper, "Incidence of diabetes and prediabetes by sickle cell trait status in African Americans," we examined whether or not this underestimation of glycemia in African Americans with SCT was impacting identification of incident cases of diabetes and prediabetes. We found that, among African Americans with SCT compared to those without, the 10-year incidence of diabetes and prediabetes was significantly lower when using hbA1c.
Overall, these findings suggest that hbA1c may not represent the best tool for screening and diagnosing diabetes in African Americans, particularly for those with SCT or whose SCT status is unknown. African Americans are already at increased risk of developing diabetes and a number of diabetic complications as compared to Whites. Using a clinical marker that may not accurately reflect glycemia places this group at an even greater risk. Although our findings need to be interpreted with caution given the limitations of the data, a reasonable alternative to relying solely on hbA1c in this population would be using multiple markers of glycemia (such as hbA1c along with fasting or 2-hour glucose) or simply using traditional glucose-based measures until further research is conducted.
INTRODUCTION


Bonora E, Tuomilehto J. The pros and cons of diagnosing diabetes with A1C. Diabetes Care. 2011; 34(S2), S184-S190.


CHAPTER ONE


CHAPTER TWO


CHAPTER THREE


