ARIC Manuscript Proposal # 2971

1.a. Full Title: Investigating the role of sickle cell trait on the association between hemoglobin A1c and other measures of glycemia in African Americans

b. Abbreviated Title (Length 26 characters): Sickle cell trait and A1c

2. Writing Group:

Writing group members (in no particular order):

Gregory Wellenius, Dept. of Epidemiology, Brown University, Providence, RI, gregory_wellenius@brown.edu
Charles Eaton, Memorial Hospital of Rhode Island, Pawtucket, RI, cbeaton51@gmail.com
Annie Gjelsvik, Dept. of Epidemiology, Brown University, Providence, RI, annie_gjelsvik@brown.edu
Xi Luo, Dept. of Epidemiology, Brown University, Providence, RI, xluo@stat.brown.edu
Wen-Chih Wu, VA Medical Center, Providence, RI, Wen-Chih.Wu@va.gov
Rakhi Naik, Dept. of Medicine, Division of Hematology, Johns Hopkins University, Baltimore, MD, rakhi@jhmi.edu
Vimal K. Derebail, University of North Carolina Kidney Center, Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Abhijit V. Kshirsagar, Division of Nephrology and Hypertension, Department of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA
Elizabeth Selvin, Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore MD, eselvin@jhu.edu

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. ______
[please confirm with your initials electronically or in writing]

First author: Mary E. Lacy, MPH
Address: 121 S. Main St., Box G, Providence, RI 02906

Phone: 214-803-7713 Fax: 
E-mail: mary_lacy@brown.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Elizabeth Selvin
Address: 2024 E. Monument St, Suite 2-600

Phone: 410-955-0495 E-mail: eselvin@jhu.edu
3. **Timeline:**

If approved, this manuscript would be part of a doctoral dissertation proposal. After receiving the data, we anticipate the following timeline: data analyses (6 months), manuscript preparation (9 months).

4. **Rationale:**

Hemoglobin A1c (A1C) reflects long-term exposure (2-3 month) to glucose in the blood. For decades it has been used to monitor glycemic control in individuals with diabetes mellitus. Since 2010, A1C has been recommended, along with fasting and 2-hour glucose, to diagnose diabetes. A1C offers a number of advantages over fasting and 2-hour glucose: it does not require fasting, is not affected by acute perturbations, has greater pre-analytical stability and lower within-person variability than fasting and 2-hour glucose, and is strongly associated with chronic complications. However, there are disadvantages to the A1C test as well: it is more expensive than fasting glucose, and there are factors that interfere with accurate A1C measurement (such as the presence of hemoglobin variants) and factors that affect interpretation of A1C results (any condition that shortens erythrocyte survival or decreases mean erythrocyte age).

Sickle cell trait (SCT) is the most prevalent hemoglobin variant in the US occurring in 8-10% of African Americans. The NGSP (formerly the National Glycohemoglobin Standardization Program) has done extensive work to identify A1C assays that provide accurate measurement of A1C even in the presence of SCT. However, there is previous, albeit limited, evidence that may suggest a shorter lifespan for red blood cells in individuals with SCT, since these individuals have approximately 30-40% hemoglobin S. It remains unclear if this may affect the degree of hemoglobin glycation, which in turn, would alter the interpretation of A1C in relationship to the glycemia values they intend to represent. Correct interpretation of A1C values in individuals with SCT is crucial as it directly impacts efforts that use A1C for screening, diagnosis and monitoring of diabetes and pre-diabetes states.

In a recent study pooling data on African Americans from CARDIA and the Jackson Heart Study, we found that SCT modifies the association between A1C and other measures of hyperglycemia. At a given glucose value (fasting or 2-hour glucose), A1C values were significantly lower in African Americans with SCT compared to those without. These A1C differences were larger at higher fasting and 2-hour glucose values. We also found that the discrimination of A1C to identify prediabetes and diabetes (defined by: fasting glucose alone, 2-hour glucose alone, and both fasting and 2-hour glucose) was lower in African Americans with SCT than those without. Although these results are based on an assay that is not reported to experience clinically significant interference from SCT, these analyses should be replicated in another setting with a different assay to determine if this finding is the result of a biological phenomenon such as shortened red blood cell survival in those with SCT which would impacts interpretation of A1C results or if this is an example of assay interference resulting from the presence of HbS.

Given our finding that SCT may influence interpretation of A1C coupled with the growing interest in non-traditional markers of hyperglycemia, we also will examine if SCT modifies the association of fructosamine and glycated albumin with fasting glucose. Fructosamine and glycated albumin reflect average glucose levels over the preceding 2-4 weeks. They are based on glycation of serum proteins and are, thus, not affected by erythrocyte or hemoglobin characteristics.

Accordingly, the objectives of this study are: 1) to determine if the type of A1C assay modifies the association between fasting glucose and A1C, 2) to determine if the association between fasting glucose and other glucose measures (fructosamine and glycated albumin) differs by SCT status, and 3) to examine if the discriminative ability of A1C, fructosamine and glycated albumin to identify diabetes (defined by fasting glucose ≥126 mg/dL, self-reported history of diabetes based on a physician diagnosis or use of glucose-lowering medications) differs by SCT status.
5. Main Hypothesis/Study Questions:

**Question 1:** For a given level of fasting glucose, is A1C significantly lower in those with SCT then those without across three NGSP-certified A1C assays?
Objective 1a: To examine the association between A1C and fasting glucose by SCT status across three different NGSP-certified A1C assays that do not experience clinically significant interference from HbS in those with SCT. Hypothesis: We hypothesize that, across all three assays, for a given fasting glucose value, A1C will be significantly lower in individuals with SCT than those without.

**Question 2:** Does SCT modify the association between fasting glucose and other glucose measures?
Objective 2a: To determine if the association between fasting glucose and A1C differs by SCT status. Hypothesis: We hypothesize that, at the same fasting glucose value, fructosamine levels will not differ by SCT status.

Objective 2b: To determine if the association between glycated albumin and fasting glucose differs by SCT status. Hypothesis: We hypothesize that, at the same fasting glucose value, glycated albumin levels will not differ by SCT status.

**Objective 3:** Does the discriminative ability of A1C, fructosamine and glycated albumin to identify prevalent diabetes differ by SCT status?
Objective 3a: To determine if the discriminative ability of A1C to identify prevalent diabetes differs by SCT status. Hypothesis: We hypothesize that the area under the receiver operating characteristic curve (AUC-ROC) of A1C to identify the presence of diabetes will be lower in African Americans with SCT than without.

Objective 3b: To determine if the discriminative ability of fructosamine to identify prevalent diabetes differs by SCT status. Hypothesis: We hypothesize that the area under the receiver operating characteristic curve (AUC-ROC) of fructosamine to identify the presence of diabetes will be lower in African Americans with SCT than without.

Objective 3c: To determine if the discriminative ability of glycated albumin to identify prevalent diabetes differs by SCT status. Hypothesis: We hypothesize that the area under the receiver operating characteristic curve (AUC-ROC) of glycated albumin to identify the presence of diabetes will be lower in African Americans with SCT than without.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

**Exclusions:** Not African American, no sickle cell variant data available, hemoglobin SC, SS, or CC
**Inclusions:** All visits at which A1C and fasting glucose (Objective 1 and 3a), fructosamine and fasting glucose (Objective 2a and 3b), and glycated albumin and fasting glucose (Objective 2b and 3c) were measured concurrently

**Outcome variables:** A1C (Objective 1), fructosamine (Objective 2a), glycated albumin (Objective 2b), prevalent diabetes (Objective 3, defined as fasting glucose ≥126 mg/dL, self report of a physician diagnosis or current use of diabetes medications)

**Main exposure:** Fasting glucose
**Modifier:** SCT
**Covariates:** Age, gender, BMI, waist circumference, eGFR, ferritin, hemoglobin
Summary statistics characterizing the study sample at visit 2 (1990-1992) will be calculated for the overall sample as well as stratified by SCT status. To examine the association between fasting glucose and A1C by SCT, we will include all visits at which fasting glucose and A1C were measured concurrently, stratified by assay. Available assays include the Tosoh 2.2 (high performance liquid chromatography method used to measure A1C at Visit 2 from 2002-2003) and the Tosoh G7 (high performance liquid chromatography method used at Visit 2 from 2007-2008 and at Visit 5), and the Tina-quant II (immunoassay method measured on a subset of participants that were enrolled in the Carotid Magnetic Resonance Imaging substudy from 2004-2005). Within assay, we will examine the association between fasting glucose and A1C using scatterplots and Pearson’s correlation coefficients, overall and stratified by SCT. We will also examine the distribution (mean and median) of A1C across categories of fasting glucose by SCT status and compare differences using the Wilcoxon rank sum test. Then, we will fit regression models to examine the association between fasting glucose and A1C including SCT*fasting glucose as an interaction term and in subgroups stratified by SCT status. We will also fit multivariable regression analyses controlling for age, gender, BMI waist circumference, eGFR, ferritin and hemoglobin to control for any potential confounding. For all regression models in which any individuals contribute more than one observation (i.e. fasting glucose and A1C measured at Visit 2 and Visit 5) we will use generalized estimating equations using a correlation matrix that accounts for within person correlation of measures. We will perform sensitivity analyses in subgroups of participants with a self-report of a physician diagnosis of diabetes or current use of diabetes medications.

We will carry-out similar analyses to examine the association between: 1) fasting glucose and fructosamine and 2) fasting glucose and glycated albumin by SCT status.

Finally, we will examine the diagnostic performance of A1C, fructosamine and glycated albumin to identify diabetes as defined by fasting glucose ≥126 mg/dL, self report of a physician diagnosis or current use of diabetes medications. We will calculate area under the receiver operating characteristic (AUC-ROC) curves for each measure of hyperglycemia in those with and without SCT and conduct unpaired comparisons of the AUC-ROC curves to assess the discriminatory power of each measure by SCT status.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes _____ No

b. If Yes, is the author aware that the file LTCDER03 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used? __X__ Yes _____ No
(This file LTCDER has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __X__ Yes _____ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER03 must be used to exclude those with value RES_DNA = “No use/storage DNA”? __X__ Yes _____ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscb.unc.edu/ARIC/search.php

______ Yes ________ No
10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data?
    ___X_ Yes  _____ No

11.b. If yes, is the proposal
    ____  A. primarily the result of an ancillary study (list number* _________)
    ____  B. primarily based on ARIC data with ancillary data playing a minor role (usually control
    variables; list number(s)* __________ _________ _________)

Selvin HbA1c ARIC Ancillary #: 2006.15
SCT ARIC Ancillary #:2263

*ancillary studies are listed by number at http://www.csc.unc.edu/aric/forms/

12a. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

12b. The NIH instituted a Public Access Policy in April, 2008 which ensures that the public has access to the published results of NIH funded research. It is your responsibility to upload manuscripts to PUBMED Central whenever the journal does not and be in compliance with this policy. Four files about the public access policy from http://publicaccess.nih.gov/ are posted in http://www.csc.unc.edu/aric/index.php, under Publications, Policies & Forms. http://publicaccess.nih.gov/submit_process_journals.htm shows you which journals automatically upload articles to Pubmed central.
References


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