1.a. **Full Title:** Short-term variability of markers of hyperglycemia

b. **Abbreviated Title (Length 26 characters):** Glycemic marker variability

2. **Writing Group:**
   Writing group members: Christina M. Parrinello; Pamela L. Lutsey; John H. Eckfeldt; Josef Coresh; David Couper; Elizabeth Selvin; others welcome

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

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3. **Timeline:** Upon approval of this manuscript proposal, we plan to complete analyses and submission of the manuscript within one year.
4. Rationale:

Fasting glucose and hemoglobin A1c (HbA1c) are traditional markers of hyperglycemia used in the diagnosis of diabetes and pre-diabetes. HbA1c is the primary test used for monitoring glycemic control in persons with diabetes. There has been recent interest in the clinical utility of nontraditional markers of hyperglycemia (fructosamine, glycated albumin and 1,5-anhydroglucitol [1,5-AG]) for diagnosis, prognosis and management of diabetes. However, the variability of nontraditional markers of hyperglycemia has not been well-studied. Older studies of these nontraditional markers have reported relatively high within-person variability. However, we suspect this is largely due to older, less accurate assays, that were not comparable across laboratories. Assays that are currently available and were used for this study have since improved.

High within-person variability, or random fluctuations around a set point, can lead to false positive results at the individual level, and substantial overestimates of disease prevalence on a population level, especially if the biomarker is only measured once. Fasting glucose has a higher within-person coefficient of variation compared to HbA1c, and has been shown to overestimate prevalence of diabetes by 24% if using one measurement as compared to two consecutive measurements. Using only one measurement and not accounting for random error in measurement can also lead to regression dilution, which can result in associations with outcomes that are biased toward the null, or weaker than the “true” association.

Quantifying the amount of within-person short-term variability inherent in these markers of hyperglycemia will allow us to understand the extent of potential misclassification of pre-diabetes/diabetes and glycemic control in epidemiologic studies and possibly in the use of these biomarkers in diagnosis and monitoring of disease status and treatment. It is also possible that measurements from this study and resulting estimates of short-term variability could be incorporated into regression models, i.e. regression calibration which allows for the simultaneous correction of variation or measurement error in exposure(s) as well as accounts for the relationship of that variation with other risk factors, and could have a substantial effect on estimates of association.

This study will enable us to quantify the variability of fasting glucose, HbA1c, fructosamine, glycated albumin and 1,5-AG over 4-8 weeks. It is crucial to understand the biological variability of these markers that have potential clinical utility for monitoring glycemic control in persons with diabetes, and may also be useful for assessing risk of diabetes. Indeed, the fact that we will conduct this study in a group of older adults is especially relevant given the high prevalence and incidence of pre-diabetes and diabetes in this age group.
5. Main Hypothesis/Study Questions:

Aim: To quantify the short-term within-person variability of fasting glucose, HbA1c, fructosamine, glycated albumin and 1,5-AG in a sample of approximately 200 participants in the Atherosclerosis Risk in Communities (ARIC) Study who were included in a repeatability study 4-8 weeks after the original visit 5 examination. Given that the degree of variability may differ across the range of biomarker measurements, secondary analyses will be conducted stratifying by diabetes status.

Hypothesis: Fasting glucose has the highest within-person variability and HbA1c has the lowest within-person variability. Fructosamine and glycated albumin have moderate within-person variability. In persons with diabetes, 1,5-AG has greater within-person variability compared to HbA1c, fructosamine and glycated albumin.

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).

Study Design and Study Population

We will include the 200 participants (50 from each field center) who attended the visit 5 exam and were asked to return for a repeat visit 4-8 weeks following the initial visit.

Laboratory Measurements

Fasting glucose was measured from stored plasma at Baylor College of Medicine on the Beckman Olympus 480 autoanalyzer using an enzymatic method. HbA1c was measured using stored whole blood at the University of Minnesota (UMN) on the Tosoh G7 automated analyzer using a high performance liquid chromatography method. Fructosamine, glycated albumin, and 1,5-AG were measured from stored serum at UMN on the Roche Cobas 6000 (using a colorimetric method for fructosamine and an enzymatic method for glycated albumin and 1,5-AG).

Statistical Analysis:

We will compare measurements of each marker obtained at the two time points in the 200 participants. We will examine descriptive statistics and calculate the mean difference between the measures. To visually compare these two measures, we will create scatterplots and Bland-Altman plots. We will also calculate the following measures to quantify the variability of each of the markers:
1) Pearson correlation coefficient; 2) within-person coefficient of variation; 3) intraclass correlation coefficient or reliability coefficient; 4) reference change value; 5) index of individuality.

To calculate some of the aforementioned measures, we will use one-way analysis of variance to obtain the within-subject and between-subject variances. We will also obtain the analytical variance from the lab’s internal quality control.

Sensitivity Analyses:

To assess differences in variability by time between measurements, we will additionally stratify analyses by time between the initial and repeat visit (e.g. <4 weeks versus ≥4 weeks). We will also stratify by diabetes status (diagnosed diabetes versus no diagnosed diabetes) to assess potential differences in variability over different ranges of values these markers.

Limitations

We may have limited precision to estimate variability of these markers in proposed stratified analyses, depending on the sample size in each stratum. Nonetheless, it will be important to describe potential differences in variability of these markers in subgroups.

REFERENCES


7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes  ____ No  
   b. If Yes, is the author aware that the file ICTDER03 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?  ____ Yes  ____ No  
   (This file ICTDER 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?  ____ 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”?  
____ 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.cscce.unc.edu/ARIC/search.php

__X__ 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)?

Previously published variability studies using ARIC data are most relevant to this study,
including:

Eckfeldt JH et al. Short-term, within-person variability in clinical chemistry test results.
Experience from the Atherosclerosis Risk in Communities Study. Arch Pathol Lab Med.
1994.

Agarwal SK et al. Sources of variability in measurements of cardiac troponin T in a
2011.

Bower JK et al. Three-year variability in plasma concentrations of the soluble receptor
for advanced glycation end products (sRAGE). Clin Biochem. 2014.

Ma J et al. Short- and long-term repeatability of fatty acid composition of human plasma

Chambless LE et al. Short-term intraindividual variability in lipoprotein measurements:

Chambless LE et al. Short-term intraindividual variability in hemostasis factors. The
ARIC Study. Atherosclerosis Risk in Communities Intraindividual Variability Study. Ann

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
__X__ A. primarily the result of an ancillary study (list number* 2014.13)
B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* _________ _________ _________)

*ancillary studies are listed by number at [http://www.cscc.unc.edu/aric/forms/](http://www.cscc.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/](http://publicaccess.nih.gov/) are posted in [http://www.cscc.unc.edu/aric/index.php](http://www.cscc.unc.edu/aric/index.php), under Publications, Policies & Forms. [http://publicaccess.nih.gov/submit_process_journals.htm](http://publicaccess.nih.gov/submit_process_journals.htm) shows you which journals automatically upload articles to Pubmed central.