1. **Full Title**: The association between morning salivary cortisol, cardiovascular risk factors, and carotid atherosclerosis: The ARIC Carotid MRI Study

2. **Abbreviated Title (Length 26 characters)**: Salivary cortisol and atherosclerosis

3. **Writing Group**: Sherita Hill Golden, MD, MHS; Gary Wand, MD; Josef Coresh, MD, PhD; Saurabh Malhotra, MD, MPH; Bruce Wasserman, MD

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**: Sherita Hill Golden

**Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author)**: Same

4. **Rationale**:

   Obesity, type 2 diabetes, and cardiovascular disease continue to be major public health burdens and type 2 diabetes is rising in epidemic proportions. Thus, identification of novel risk factors for these diseases is important in guiding the development of preventive interventions. Psychological stress, particularly depression, has been shown to be a risk factor for the development of both type 2 diabetes (1-5) and cardiovascular disease (6-9) but the mechanism remains unclear. Neuroendocrine changes induced by these psychological factors, specifically activation of the hypothalamic-pituitary-adrenal (HPA) axis, might provide a unifying explanation. Dysregulation of the HPA axis has been documented in individuals with various forms of psychological stress, including depression (10-13), anxiety(14), history of childhood abuse (15) (16,17)and/or adult trauma (17)and post-traumatic stress disorder(18). Neuroendocrine dysfunction has also been documented in obesity (19-22)and diabetes(23). Cross-sectional studies assessing the relation of HPA axis activity, by measurement of salivary cortisol, and cardiovascular risk factors have demonstrated a positive association between morning cortisol and cardiovascular risk factors, such as blood pressure, lipids, glucose, and intimal-medial thickness reactivity (24-26), although one study found no association between salivary cortisol response to awakening and the metabolic syndrome(27). With the exception of one study of 509 South Asian individuals, these studies were limited by small samples sizes and by being performed primarily in Caucasians in Europe.
Our Salivary Cortisol Ancillary Study to the ARIC Carotid MRI Study, provides a unique opportunity to assess the association of salivary cortisol, as a proxy of hypothalamic-pituitary-adrenal axis activity, with multiple cardiovascular risk factors and carotid atherosclerosis that have been assessed thoroughly, uniformly and in a large, bi-racial cohort.

5. Main Hypothesis/Study Questions:

1. Is morning salivary cortisol associated with prevalent coronary heart disease (CHD) and prevalent type 2 diabetes mellitus?

2. Among individuals without prevalent type 2 diabetes, is morning salivary cortisol associated with cardiovascular risk factors, including adiposity (waist circumference, body-mass index), fasting plasma glucose, fasting plasma insulin, dyslipidemia (triglycerides, HDL-cholesterol), and systolic and diastolic blood pressure?

3. Is morning salivary cortisol associated with carotid wall thickness and the presence and characteristics of carotid plaque, assessed by carotid MRI?

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: Cross-sectional analysis

Data Variables: Age, race/ethnicity, gender, ARIC site, prevalent CHD, prevalent type 2 diabetes mellitus, prevalent hypertension, fasting plasma glucose, fasting plasma insulin, lipids (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides), systolic blood pressure, diastolic blood pressure, smoking status, dietary intake, physical activity, medications (cholesterol lowering, anti-hypertensive therapy), adiposity (waist circumference, body-mass index), carotid wall thickness (mean and maximum), presence of carotid plaque, lipid core size (as proxy of plaque vulnerability), plaque contrast enhancement (as proxy of inflammation and neovascularization), and morning salivary cortisol.

Brief analysis plan and methods

Table 1. Outline of analysis plan.

<table>
<thead>
<tr>
<th>Markers of HPA Axis Activation</th>
<th>Consequences of Neuroendocrine Activation</th>
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<tbody>
<tr>
<td>8 am salivary cortisol</td>
<td>Visceral obesity</td>
</tr>
<tr>
<td></td>
<td>Hypertension</td>
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<tr>
<td></td>
<td>Glucose intolerance/insulin resistance</td>
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<td></td>
<td>Dyslipidemia</td>
</tr>
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<td>Atherosclerosis</td>
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</tbody>
</table>

Confounders/Mechanisms: Diet, physical activity, smoking status, obesity
All analyses will be conducted by incorporating the sampling weights. (1) Correlation analyses and linear regression models will be used to determine the correlation between morning salivary cortisol and the following measures: (a) adiposity, assessed by waist circumference and body-mass index, (b) fasting glucose, (c) fasting insulin, (d) lipid parameters (total cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol), and (e) blood pressure.

(2) Correlation analyses and linear regression models will be used to determine the correlation between morning salivary cortisol and the following measures of carotid atherosclerosis: (a) mean and maximum carotid wall thickness, (b) lipid core size (as a proxy of plaque vulnerability), and (c) plaque contrast enhancement (as a proxy of inflammation and neovascularization). Analyses will be performed to look for differences in correlation between cortisol and WT between individuals with lipid core with those without it. Subgroup analyses will be performed by the different IMT groups.

Individuals with diabetes will be excluded since treatment of glycemia may confound the association between salivary cortisol and fasting glucose. Subsidiary analyses will be conducted in individuals with and without prevalent cardiovascular disease to determine if the associations differ in these two populations. In multivariate analyses, we will be able to adjust for potential confounders of the association between morning cortisol and metabolic risk factors using data already collected in the study on demographics, diet, physical activity, smoking status, and medication use. For analyses involving the MRI variables only the studies with sufficient image quality and those with adherence to the MRI protocol will be included.

**Anticipated methodological limitations:**

We have reviewed quality control data from the salivary cortisol samples which was not available at the time of the initial proposal submission. While the reproducibility of the assay was good on split replicate samples from the same salivette ($R^2=0.8945$), the reproducibility between salivettes on a given visit or between different visits was poor ($R^2=0.22$; coefficient of variation=47.4%). Only a few small studies have assessed the repeatability of salivary cortisol measures over time. Two studies with 20-30 healthy individuals each found salivary cortisol measurements assessed several weeks apart (1-10 weeks) were more variable than plasma cortisol levels (28, 29) and in one of these studies, the intraclass correlation coefficient was 0.18 for 8 am salivary cortisol (28). A more recent study, however, found a much stronger correlation of 0.41 (p-value <0.001) between 8 am baseline salivary cortisol and 8 am repeated salivary cortisol assessed at 6 months among 75 individuals without major depressive disorder (30).

We recognize that the poor reproducibility of salivary cortisol will be the major limitation to our study and this will be clearly stated in the discussion of the manuscript, whether we find positive or null associations. Our prior work suggests that more integrative measures of HPA axis activity may be better markers of its activity than single point-in-time measures given the dynamic nature of the HPA axis (31). Also, the dynamics of the HPA axis may very depending on the presences of co-morbid conditions, such as diabetes and cardiovascular disease. We will request to examine the salivary
cortisol quality control data in a separate manuscript proposal as this information will be helpful in determining how to examine the HPA axis in future epidemiological studies.

**Conclusion:**

ARIC provides a unique opportunity to examine the association of salivary cortisol, as a proxy of hypothalamic-pituitary-adrenal axis activity, with multiple cardiovascular risk factors and carotid atherosclerosis that have been assessed thoroughly, uniformly and in a bi-racial cohort. To our knowledge, there are no large epidemiological studies that have examined these hypotheses previously. The addition of this hormonal assessment has placed ARIC in a position to expand the field of cardiovascular epidemiology to include less traditional hormonal measurements.

7.a. Will the data be used for non-CVD analysis in this manuscript? _x_ Yes _x_ No
b. If Yes, is the author aware that the file ICTDER02 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 ICTDER02 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 _x__ No

8.b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”? _____ Yes _____ No N/A

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.cscc.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)?

There are none.
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* 2005.11)
____ 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/

12. 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.


22. Bjorntorp P, Rosmond R: Obesity and cortisol [In Process Citation]. *Nutrition* 16:924-936, 2000


