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

b. Abbreviated Title (Length 26 characters): Depression, salivary cortisol, and atherosclerosis

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

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

3. Timeline: To begin immediately once salivary cortisol and depression data have been entered at the Coordinating Center and forwarded to Dr. Golden

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).

Most studies of the association between depression and metabolic outcomes have not included measurement of HPA axis activity to determine if this was one potential mechanism linking the two. Exceptions are two recent cross-sectional studies. In the Heart and Soul Study, depressed subjects with coronary heart disease had higher 24-hour UFC levels than coronary heart disease subjects without major depression(24). In
another study, depressed postmenopausal women with hypercortisolism had increased accumulation of intra-abdominal fat and higher glucose levels during an oral glucose tolerance(25). Neither study used regression techniques to determine if hypercortisolism explained the association between depression and metabolic outcomes.

Our Salivary Cortisol Ancillary Study to the ARIC Carotid MRI Study, provides a unique opportunity to examine the association between depressive symptoms, assessed by the Center for Epidemiological Studies Depression (CES-D) scale, and cardiovascular risk factors and carotid atherosclerosis that have been assessed thoroughly, uniformly and in a large, bi-racial cohort. Because we have data on morning salivary cortisol, as a proxy of HPA axis activity, we will be able to determine if it is an explanatory factor in the association between depressive symptoms and cardiovascular risk factors and atherosclerosis.

5. Main Hypothesis/Study Questions:

1. Are depressive symptoms associated with prevalent coronary heart disease (CHD) and prevalent type 2 diabetes mellitus?
   a.) Is this association fully or partially explained by morning salivary cortisol?

2. Among individuals without prevalent type 2 diabetes, are depressive symptoms 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?
   a.) Is this association fully or partially explained by morning salivary cortisol?

3. Are depressive symptoms associated with carotid wall thickness and the presence of and characteristics of carotid plaque, assessed by carotid MRI?
   a.) Is this association fully or partially explained by morning salivary cortisol?

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), morning salivary cortisol, CES-D score.
**Brief analysis plan and methods**

Table 1. Outline of analysis plan.

<table>
<thead>
<tr>
<th>Psychological Stress</th>
<th>Consequences of Neuroendocrine Activation</th>
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</thead>
<tbody>
<tr>
<td>Depressive symptoms:</td>
<td>Visceral obesity</td>
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<tr>
<td>Continuous</td>
<td>Hypertension</td>
</tr>
<tr>
<td>Categorical (CES-D≥16)</td>
<td>Glucose intolerance/insulin resistance</td>
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<tr>
<td></td>
<td>Dyslipidemia</td>
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<td></td>
<td>Atherosclerosis</td>
</tr>
</tbody>
</table>

Confounders/Mechanisms: **8 am salivary cortisol** and others (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 CES-D (modeled as a continuous variable) 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. Linear regression will also be used to determine the mean difference in these variable between individuals with depressive symptoms (CES-D≥16) and those without depressive symptoms (CES-D<16).

(2) Correlation analyses and linear regression models will be used to determine the correlation between CES-D 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). Linear regression will also be used to determine the mean difference in these variable between individuals with depressive symptoms (CES-D≥16) and those without depressive symptoms (CES-D<16).

In both of these analyses, adjustment will be made for morning salivary cortisol to determine if it explains the association between depressive symptoms and cardiovascular risk factors. In additional multivariable models, adjustment will be made for demographics, diet, physical activity, smoking, and medication use.

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.

**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 (26, 27) and in one of these 
studies, the intraclass correlation coefficient was 0.18 for 8 am salivary cortisol (26). 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 (28).

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 (29). 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? __Yes__ No 
7.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 __ 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
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

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


