ARIC Manuscript Proposal # 3366

PC Reviewed: 3/12/2019	**Status: _____**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Endogenous Dehyroepiandrosterone sulfate (DHEA-S) Levels and Cardiovascular Health in Older Adults – Atherosclerosis Risk in Communities Study (ARIC)

b. Abbreviated Title (Length 26 characters): DHEA-S and CV Health

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

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3. Timeline: Analysis is anticipated to begin as soon as approval is obtained. The manuscript is to be prepared as soon as analyses are available. The analysis and manuscript preparation are anticipated to take place within one year of approval of the proposal.

4. Rationale:

Circulating concentrations of steroid hormone dehyroepiandrosterone (DHEA) and its sulfate, DHEA-S, decline dramatically with age and low DHEA/-s levels have been shown to be associated with cardiometabolic disease and mortality in older adults (1-3). Proposed cardioprotective mechanisms of DHEA based on in vitro and animal studies include effect on endothelial function, vascular remodeling, insulin resistance and inflammation. Lower levels of DHEA/DHEA-S have been shown to have association with higher progression of coronary and aortic atherosclerosis as well as carotid intimal-medial thickness (4-6). Lower levels of DHEA/DHEA-S have further been shown to be associated with cardiovascular disease (CVD) and mortality in older men and women, though results have been inconsistent (7-11). Moreover, previous randomized control trials of DHEA supplementation have failed to show clear clinical benefit (cite).

Though there is a large volume of epidemiological data on the association between DHEA/DHEA-S and CVD, especially pertaining to atherosclerotic cardiovascular disease (ASCVD) and mortality, the association of DHEA/DHEA-S levels with subclinical CVD and heart failure in older adults has been largely unexplored. Moreover, understanding of the mechanism of how DHEA/-S concentration is associated with CVD, whether through environmental versus genetic factors remains unclear. Furthermore, the associations of DHEA/DHEA-S and CVD may differ between women and men.

The Atherosclerosis Risk in Community (ARIC) study incorporates a large sample size, with DHEA-S measurements at multiple timepoints, broad collection of cardiovascular risk factors, rich biomarker data for the characterization of subclinical disease as well as rigorous adjudication of outcome events. Therefore, we feel that it will be important to evaluate DHEA-S and its association with cardiovascular health in older men and women, including assessment of relationship to subclinical CVD and heart failure as well as investigation of environmental and genetic impact on DHEA-S levels.

5. Main Hypothesis/Study Questions:

Hypothesis: DHEA-S concentration is significantly associated with cardiovascular health, including subclinical CVD and heart failure in older men and women. Higher DHEA-S levels are associated with "healthy cardiovascular aging" and low levels are associated with subclinical and clinical CVD. There may be differences in association by sex with more association between low DHEA-S levels and CVD outcomes in men. DHEA-S levels in older adults are influenced by both environmental as well as genetic factors.

Study questions:

1. What is the association between DHEA-S concentration with "healthy cardiovascular aging", subclinical and clinical CVD in older men and women?

2. In older men and women without known clinical CVD, does low DHEA-S concentration have association with risk for incident CVD events and mortality?

3. Do associations of DHEA-S and CVD differ by sex?

4. Does change in DHEA levels with aging have association with risk for incident CVD events and mortality?

5. Are there genetic variants associated with serum DHEA-S concentration and if so, are these variants also associated with cardiovascular health in older adults?

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 methodological limitations or challenges if present).

Study Design:

In this study, ARIC visit 5 will serve as the baseline visit for the primary analysis [mean (SD) age 75.6 (5.26) for women and 76.0 (5.22) for men]. All women at ARIC visit 5 were post-menopausal. For cross-sectional analysis assessing the association between DHEA-S and cardiovascular health status at visit 5, we will exclude participants without DHEA-S measurements, those without hs-TnT or NT-proBNP measurements as these biomarkers were used as the manifestation of subclinical disease, those of race other than white or African American as well as non-white participants at the Minneapolis and Washington County centers. In prospective analysis assessing the association of DHEA-S level at visit 5 with incident CVD events, further exclusions will be applied to participants with prevalent CHD, those with prevalent stroke, and those with prevalent HF**.** In an exploratory analysis, we will also assess whether DHEA-S levels at an earlier timepoint is associated with incident CVD events.

For this exploratory analysis, ARIC visit 4 [mean (SD) age 62.4 (5.60) for women and 62.9 (5.67) for men] will serve as the baseline visit. Similar exclusion criteria will be applied to this exploratory analysis.

Exposure:
The primary exposure variable in this study will be DHEA-S concentration at visit 5. DHEA-S concentration will be modeled both as a continuous variable (natural log transformed) and as a categorical variable by tertiles. All analysis will be performed separately for men and women. In exploratory analyses, we will also use DHEA-S concentration at visit 4, also modeled as a continuous and categorical variable. We will further perform exploratory analysis using the change of DHEA-S between visit 4 and visit 5 as the exposure variable.

Co-variates:
Co-variates of interest include: age, race-center, total cholesterol, HDL cholesterol (HDL-C), systolic blood pressure (SBP), use of antihypertensive medication, smoking status, diabetes status, body mass index (BMI), estimated glomerular filtration rate (eGFR) and history of hormone therapy (HT) use (women only). Medical history, demographic data, anthropometric data, blood pressure measurements, and lipid measurements will be obtained at ARIC visit 5 (or at ARIC visit 4 in exploratory analysis using visit 4 as the baseline visit) with the exception of HT use. HT data are available at ARIC visit 4 but not at visit 5. We will include adjustment for a history of HT use as a sensitivity analysis by carrying over data from visit 4.

Outcome measures:
For cross-sectional analysis, cardiovascular health status at visit 5 will be categorized as clinical CVD, subclinical CVD and no CVD. Clinical CVD will be defined as prevalent coronary heart disease (CHD), stroke or heart failure (HF) at or prior to visit 5. Subclinical disease will be defined as having either visit 5 hs-TnT or NT-proBNP concentrations above the lowest biomarker tertile specified by sex. For hs-TnT, the lowest tertile cutpoint corresponds to 11 ng/L for men and 7 ng/L for women. For NT-proBNP, the lowest tertile cutpoint corresponds to 77.39 pg/mL for men and 96.26 pg/mL for women. Finally, no CVD status at visit 5 will be defined as the absence of known clinical CVD and low biomarker evidence of subclinical disease (both hs-TnT, and NT-proBNP levels below their respective cutpoints). As androgens may exert a suppressive effect on natriuretic peptides(12, 13), we will perform a sensitivity analysis using hs-TnT (and not NT-proBNP) for subclinical disease.

For prospective analysis, the outcome measures will be post-visit 5 incident CVD events or death (or post-visit 4 events in exploratory analysis). Outcomes measures evaluated in this study were incident CHD, incident stroke, incident HF hospitalization, all-cause mortality, incident ASCVD, and incident global CVD. ASCVD was defined as the composite of incident CHD and incident ischemic stroke; global CVD was defined as the composite of incident CHD, incident ischemic stroke, and incident HF hospitalization.

Statistical analysis:

All analysis will be performed separately for men and women. First, we will perform multivariate regression analysis to assess independent association between co-variables and level of DHEA-S at visit 5.

Cross-sectional association between visit 5 log-transformed DHEA-S and cardiovascular health status will be evaluated using multinomial logistic regression models with stepwise adjustment.

Model 1 will adjust for age and race-center
Model 2 = model 1 plus total cholesterol, HDL-C, SBP, use of antihypertensive medication, smoking status, and diabetic status
Model 3 = model 2 plus BMI and eGFR
Model 4 = model 3 plus testosterone and SHBG
In sensitivity analysis, we will include a model 5 = model 4 plus history of HT use

To evaluate whether there is heterogeneity of effect by race, we will further perform analysis stratified by race.

For prospective analysis assessing DHEA-S and post-visit 5 incident CVD events or deaths (or post-visit 4 events in exploratory analysis), we will model DHEA-S as both a categorical as well as a continuous variable. For tertile analysis, the lowest tertile will be used as the reference group. For the continuous analyses, DHEA-S values will be log-transformed. Cox proportional hazard models will be used to estimate the hazard ratios (HRs) and 95% confidence intervals (CIs) for the associations between DHEA-S level and incident CHD, ischemic stroke, ASCVD, HF hospitalization, global CVD, and all-cause mortality. Adjustment models 1 through 5, as described above, will also be used in the Cox analysis. We will also perform spline analysis, modeling DHEA-S as restricted cubic splines in Cox regression analysis to assess the shape of the association between DHEA-S and outcome variables. Again, we will assess for heterogeneity by race with stratified analysis by white versus African American individuals.

Genetic analysis of variants associated with DHEA-S level and CV health status:
We propose to assess genetic variants associated with serum DHEA-S levels and association with cardiovascular health status. Previously, GWAS has been performed to determine common genetic variants associated with serum DHEA-S levels (14).

We will perform confirmatory analysis of previously described variants *ZKSCAN5, SULT2A1, ARPC1A, TRIM4, BMF, HHEX, BCL2L11, CYP2C9* and association with DHEA-S levels at ARIC visit 4 and visit 5. Upon confirming variants with DHEA-S concentrations, we will assess association between variants and cardiovascular health status (clinical CVD, subclinical CVD and no CVD) using adjusted logistic regression models.

Limitations:

- DHEA is weakly androgenic and is a precursor to sex hormones testosterone and estradiol. Though it is not known if the association between DHEA and CVD is mediated via sex hormones, some previous studies have studied DHEA together with testosterone and estradiol. The ARIC dataset includes testosterone

but does not contain information on estradiol, therefore we will not be able to adjust for estradiol in the analysis.

- Similarly, it is unclear whether HT has effect on DHEA-S levels. While ARIC has data on HT use at visit 4 (1996-1996), there is no data at visit 5 (2011-2013). After publication of the Women's Health Initiative Study in 2002, the use of HT has dramatically declined. Therefore, carrying over visit 4 HT data to visit 5 may not accurately reflect HT use at visit 5 and is why we adjust for HT use as a sensitivity analysis only.

7.a. Will the data be used for non-CVD analysis in this manuscript? ____ Yes __X_ 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?
__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.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)?

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

11.b. If yes, is the proposal
___ A. primarily the result of an ancillary study *
___ 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/

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.cscc.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.

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