ARIC Manuscript Proposal # 2234

1.a.   **Full Title**: Relationship between serum testosterone and the incidence of preclinical and clinical cardiovascular disease in men in the Atherosclerosis Risk In Communities (ARIC) cohort study

  b. Abbreviated Title (Length 26 characters): **Serum T and CVD risk**

2.   **Writing Group**:
     Writing group members: Reshmi Srinath, MD; Sherita Hill Golden, MD, MHS; Kathryn A. Carson, ScM; Adrian Dobs, MD, MHS

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

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3.   **Timeline**: Data analysis will begin immediately with expected completion within 12 months.

4.   Rationale:

The overall goal of this proposal is to understand the relationship between serum testosterone and preclinical and clinical coronary heart disease. Cross-sectional observational studies in men have demonstrated that low testosterone is associated with increased all-cause mortality and cardiovascular mortality (Malkin et al. Heart 2010). We also know that low testosterone level is prevalent in up to 30% of the male population over the age of 60 (Harman et al. JCEM 2001). Low testosterone may cause elevated coronary heart disease risk mechanistically since it is associated with lipid abnormalities, insulin resistance,obesity and proatherogenic changes on the vasculature. Carotid intima media thickness (IMT) is now considered a marker of preclinical coronary heart disease which can help predict future coronary heart disease risk. A small cross-sectional study has previously shown an inverse relationship between serum testosterone and maximal common carotid IMT in 239 middle aged men from Finland (Makinen et al. JACC 2005). However there are few prospective studies to date which have looked at the effect of serum testosterone level on the development of both pre-clinical and clinical coronary heart disease. A recent prospective case cohort study from France of ~500 men over the age of 65 suggested a J-shaped association between serum testosterone and risk of cardiovascular event or ischemic stroke, with limited follow up over four years (Soisson et al. Maturitas 2013).

   Measuring serum samples in a large sample of men using the ARIC cohort will allow us to look at the distribution of serum testosterone in males and to document the association with preclinical atherosclerosis defined by IMT and clinical atherosclerosis defined by incidence of coronary heart disease and cardiovascular events. Since testosterone therapy has grown to near $2 billion annually (IMS Health 2011) the timing is ideal to consider the role of testosterone levels in the early stages of atherosclerosis and coronary heart disease.

5.   Main Hypothesis/Study Questions:

Low serum testosterone is associated independently with the presence of preclinical atherosclerosis as defined by carotid IMT and clinical atherosclerosis as defined by incidence of coronary heart disease and cardiovascular events.

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: Prospective cohort study

Inclusion criteria: male>age 55, no prior history of coronary heart disease or ischemic stroke at baseline (ARIC Visit 4), no prior exposure to exogenous testosterone or exposure during the course of the study based on review of medications performed at baseline and follow up visits.

Exclusion criteria: females, males <age 55, known prior history of coronary disease or ischemic stroke at baseline (ARIC Visit 4), exposure to exogenous testosterone prior to or

during the course of the study as assessed by review of medications performed at baseline and follow up visits.

Data to be collected: Serum samples have been requested from frozen samples taken from participants during visit 4(1996-1999). We have requested samples taken from males before 1000 AM with sufficient volume for laboratory analysis(>0.5 cc). Serum total testosterone and sex hormone binding globulins (SHBG) using liquid chromatography mass spectrophotometry were performed in 2012 by Dr. Shallender Bhasin (Boston University, Boston, MA).

Primary outcome: Carotid IMT obtained at visit 4(1996-1999) using high resolution B-mode ultrasound. The variable for analysis is the average IMT of the far wall across the left and right common carotid, carotid bifurcation and internal carotid artery respectively(six sites total). Missing values were imputed from from sex- and race-specific multivariate linear models of mean IMT. Our other outcome is incidence of coronary heart disease and incidence of cardiovascular events with annual surveillance through 2010.

Other covariates: Age, race/ethnicity, gender, ARIC site, dietary intake, smoking status, physical activity status, medications (cholesterol lowering, anti-hypertensive therapy), adiposity (waist circumference, body-mass index), fasting glucose, fasting insulin, fasting lipids, blood pressure, diabetes status, hypertension status.

Analysis:
  (1) Correlation analyses and linear regression models will be used to determine the correlation between morning serum testosterone and the following measures: adiposity (waist circumference, body-mass index), fasting glucose, fasting insulin, lipid parameters (total cholesterol, triglycerides, HDL-cholesterol, LDL cholesterol), blood pressure.
  (2) Correlation analyses and linear regression will be used to determine the relationship between morning serum testosterone and the following measures: average IMT of the far wall on ultrasound obtained at visit 4(1996-1999).
  (3) Survival analyses will be used to assess the relationship between morning serum testosterone and incidence of coronary heart disease and cardiovascular events. Participants will be divided by gonadal status (normal versus hypogonadal total testosterone <300 ng/dL versus elevated total testosterone>900 ng/dL.) Multivariate analysis will be used to adjust for potential confounders using data already collected in the study including demographics, diet, physical activity status, smoking status and medication use.

Data analysis will be conducted using SAS version 9.3 (SAS Institute, Inc., Cary, NC)

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?** **x____ Yes ____ No**
(This file ICTDER03 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 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.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? **_X___ Yes ____ No**

11.b. If yes, is the proposal
_X__ A. primarily the result of an ancillary study (list number* 2008.01_, 2011.02_____)
___ 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.

References:
Harman SM, Metter EJ, Tobin JD et al. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. *J Clin Endocrinology Metabolism* 2001;86:724-731.

Makinen Juuso, Jarvisalo M et al. Increased carotid atherosclerosis in andropausal middle-aged men. *Journal of the American College of Cardiology* 2005;45:1603-8.

Malkin CJ et al. Low serum testosterone and increased mortiality in men with coronary heart disease. *Heart* 2010;96:1821-1825.

Soisson, Veronique et al. A J shaped association between plasma testosterone and risk of ischemic arterial event in elderly men: the French 3C cohort study. *Maturitas* 75(2013)282-288.