1.a. **Full Title:** Association between plasma testosterone, adiponectin and the incidence of metabolic syndrome among men in the Atherosclerosis Risk in Communities (ARIC) cohort study.

b. **Abbreviated Title (Length 26 characters):** T, Adiponectin and MetS

2. **Writing Group:**
   Writing group members: Swaytha Yalamanchi, MD; Sherita Hill Golden, MD,MHS; Rita Kalyani, 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. **SY** [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 endogenous sex hormones, adipokines, and the risk of metabolic syndrome. The prevalence of metabolic syndrome is increasing. A study involving 8,814 adults in the NHANES III cohort reported that the prevalence of metabolic syndrome is approximately 22% with an age-dependent increase (ranging from 6.7% in those 20-29 years of age and 42.0% in those >70 years of age). Those with metabolic syndrome have an increased risk for the subsequent development of type 2 diabetes, cardiovascular disease and all cause mortality. Prior cross-sectional and longitudinal studies have shown that low testosterone and SHBG are independently associated with the risk of metabolic syndrome, while a decreased estradiol/testosterone ratio may be associated with a reduced risk in men. Conversely, men with metabolic syndrome may be more likely to have low testosterone due to changes in body composition with increased fat mass, decreased sex hormone binding globulins, increased aromatase activity leading to testosterone conversion to estrogen and increased inflammatory mediators. Given this bi-directional relationship, more research is needed to determine how to treat men with androgen deficiency in the context of metabolic syndrome. Furthermore, the role of adipokines in mediating the risk factor profile of developing diabetes and cardiovascular profiles has been investigated. Higher levels of adiponectin have been associated with increased insulin sensitivity, lower risk of atherosclerosis and anti-inflammatory properties. Higher levels of adiponectin are associated with a lower risk of diabetes mellitus in older individuals, but its role in insulin sensitivity independent of visceral adiposity remains unclear. Furthermore, there is little data regarding the relationship of testosterone with adiponectin in the context of metabolic syndrome independent of other cardiac risk factors and changes in body composition. While there has been some evidence to suggest that higher endogenous testosterone levels are correlated with higher adiponectin levels, there has also been evidence that exogenous testosterone therapy decreases adiponectin levels.

5. **Main Hypothesis/Study Questions:**

**Aim 1:** To determine if lower serum testosterone levels are associated with an increased risk of incident and prevalent metabolic syndrome.

**Aim 2:** To determine if lower serum testosterone levels are associated with increased leptin and decreased adiponectin levels.

**Aim 3:** To determine if increased leptin and/or decreased adiponectin levels are associated with prevalent and incident metabolic syndrome.
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:** Metabolic syndrome will be analyzed cross-sectionally at visit 4 and incidentally at visit 5. A smaller subset in which we have adipokine levels (drawn at visit 1) and sex hormone levels (drawn at visit 4) will also be analyzed as part of a prospective observational study.

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

**Exclusion criteria:** Women, men <55 years, those with known prior history of coronary disease or ischemic stroke at baseline (ARIC Visit 4), exposure to exogenous testosterone or estrogen 6 months prior to or during the course of the study as assessed by review of medications performed at baseline and follow up visits. The following will be exclusion criteria in analysis of incident, but not prevalent, metabolic syndrome.

**Data to be collected:** Plasma samples for testosterone were requested from frozen samples taken from participants during visit 4. We have limited our analysis of testosterone levels to those samples taken from males before 10:30 AM with sufficient volume for laboratory analysis (>0.5 cc). Plasma total testosterone using liquid chromatography mass spectrophotometry was performed in 2012 by Dr. Shallender Bhasin (Boston University, Boston, MA). Total adiponectin was measured in duplicate by radioimmunoassay (Linco Research, St. Charles, MO) using 125I-labeled murine adiponectin as a tracer and a multispecies adiponectin rabbit antiserum for detection of adiponectin in human plasma calibrated against recombinant human adiponectin standards as previously described. 21

**Primary outcome:** Prevalence of metabolic syndrome at visit 4 and incidence of metabolic syndrome at visit 5 (2012-2013) as defined by the 2005 National Cholesterol Education Program (NCEP) III criteria with at least 3 of the following: elevated fasting glucose (≥ 100 mg/dl) or drug treatment for elevated blood glucose, low HDL (<40 mg/dl for men), hypertriglyceridemia (≥ 150 mg/dl or fibrate treatment for elevated triglycerides), increased waist circumference (≥102 cm in men) and elevated blood pressure (≥130/85 mm Hg or drug treatment for hypertension).

**Secondary outcomes:**
Relationship of serum testosterone levels with leptin and adiponectin levels.

Relationship of increased leptin and/or decreased adiponectin levels with prevalent and incident metabolic syndrome.
Other covariates: Age, race, center, alcohol use, smoking status, adiposity (BMI), insulin resistance (fasting insulin), and inflammatory markers (C-reactive protein and interleukin-6).

Analysis:
- General linear models regression (continuous measures) and chi-square tests (categorical measures) will be used to determine the association of morning plasma testosterone with the following measures: age, race/center, smoking status, adiposity (waist circumference, body-mass index), adiponectin, leptin, fasting LDL, triglycerides, HDL, inflammatory markers (C-reactive protein and interleukin-6), use of anti-lipid medications, hypertension status, anti-hypertension medications, diabetes status and hypoglycemic agents at visit 4 (baseline).
- Logistic regression analysis will be used to assess the association of morning plasma testosterone with the prevalence of metabolic syndrome at baseline (visit 4). Models will be adjusted for age, race/center, alcohol use and smoking status at visit 4.
- Logistic regression will be used to assess the association of morning plasma testosterone and incidence of metabolic syndrome at visit 5 (excluding those with metabolic syndrome at visit 4). Models will be adjusted for age, race/center, alcohol use and smoking status measured at baseline (visit 4). In sequential models we will also adjust for BMI, and body fat from visit 4 as insulin sensitivity and obesity can influence testosterone and SHBG levels. 24
- Logistic regression models will also be used to explore the association of testosterone with individual components of metabolic syndrome at baseline (visit 4) and at visit 5.
- Logistic regression models will be used to determine if increased leptin and/or decreased adiponectin levels are associated with incident and prevalent metabolic syndrome both with and without testosterone as a covariate.

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_DÑA = “CVD Research” would be used?
   ____ 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”? 

_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 list under the Study Members Area of the web site at: http://www.csc.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.csc.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.csc.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.


