ARIC Manuscript Proposal # 3304

1.a. Full Title:
Prevalence of biotin supplement use and its association with high sensitivity cardiac troponin T and NT-proBNP results in ARIC Visit 6

b. Abbreviated Title (Length 26 characters):
Biotin use and lab tests

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

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3. Timeline:
We will analyze the data within 6 months and submit the manuscript for publication in a year.

4. Rationale:
Biotin supplement use, especially in large doses, may be associated with false positive test results for some assays.¹ Concerns have been raised about the possibility that dietary biotin supplement use, especially in high doses such as 5 and 10 mg daily, may interfere with common clinical laboratory tests, causing inaccurate results.¹ In November of 2017, the US Food and Drug Administration (FDA) issued a safety communication, warning of this possibility.⁷ Biotin is a water-soluble B-vitamin found in meat, fish, eggs, and dairy. While biotin deficiencies are relatively rare,² biotin supplement use has recently become popular for presumptive health and cosmetic benefits ³, ⁴ and treating certain medical conditions.⁵ According to a recent survey, self-reported prevalence of high dose biotin supplement use (5 and 10 mg) among outpatients was 34/1944 (or 1.7%).⁶ However, presently little is known about the dose-specific prevalence of biotin supplement use in the general population in US, especially at large doses (e.g., 5 mg or higher) which may affect lab test accuracy.

Many blood-based lab tests are immunoassays that exploit the strong binding between biotin and streptavidin. Normally, biotin concentrations in blood samples are too low to cause test interference. However, consumption of biotin as a vitamin supplement or high-dose therapy (i.e., for treatment of inborn error of metabolism and multiple sclerosis) can interfere in many immunoassay methods due to the unnaturally high concentrations of biotin present in blood. The unnaturally high concentration of biotin interrupts biotin-streptavidin interaction inherent in these immunoassay designs and results in assay interferences. For example, 10 mg of biotin supplement is 333-fold higher than the daily recommended dose of 0.03 mg, which can increase blood biotin levels to 100 ng/mL, a concentration above many of known threshold of interference in immunoassay tests manufactured by many diagnostics companies (e.g., Roche Diagnostics).

We demonstrated in a cross-over trial of 6 healthy subjects that a daily biotin supplement of 10 mg daily was statistically significantly associated with potentially clinically important assay interferences in some, but not all, 23 biotinylated hormone and nonhormone assays studied.¹ Assays with evidence of interference included those for thyroid-stimulating hormone (TSH), total thyroxine, total triiodothyronine, free thyroxine, free triiodothyronine, parathyroid hormone, prolactin, N-terminal pro-brain natriuretic peptide [NT-proBNP], 25-hydroxyvitamin D, prostate-specific antigen and ferritin. However, we did not examine the impact of biotin supplementation on cardiac troponin tests, a clinically important biomarker to aid in the diagnosis of heart attacks.⁴, ⁸ Based on a recent study, 7.4% (95% CI, 6.2-8.9%) of plasma samples from a sample of patients in the emergency department had concentrations at or above the lowest known threshold (10 ng/mL) for biotin interference for all Roche's immunoassays.⁶ Furthermore, high sensitive cardiac troponin T (i.e., Roche Gen 5 cardiac troponin T) is prone to biotin interference (i.e., the design of the immunoassay utilizes biotin and streptavidin interaction) and was determined to have a threshold of biotin interference at 30 ng/mL (e.g., 10% decrease in the cardiac troponin T results, personal communication). Therefore, biotin ingestion may lead to serious clinical implications including a missed diagnosis of myocardial infarction using high sensitive cardiac troponin T.⁷ In addition, even though we demonstrated that among healthy individuals biotin supplement use at large doses falsely decreases NT-proBNP (an important biomarker used clinically to evaluate heart failure),¹ the impact of biotin supplement use on NT-proBNP in ARIC Visit 6 is unclear.

The objectives of this study are 1) to estimate the prevalence of high dose biotin supplement use (i.e., ≥5 mg daily and ≥10 mg daily) at ARIC 6; and 2) to determine cross-sectional associations between high dose biotin supplement use and 2 biotinylated assay results
(high sensitive cardiac Troponin T and NT-proBNP) at the ARIC Visit 6. We chose ARIC Visit 6 because that given that the biotin use trend is very recent, it is possible that there are not a sufficient number of users at high enough doses of biotin at visit 5 to conduct meaningful analyses of high doses. Among the lab test results available in ARIC Visit 6 as of now (12/3/18), high sensitive cardiac Troponin T and NT-proBNP are the only two lab tests that are prone to biotin interference (e.g., the design of these two immunoassay tests utilize biotin and streptavidin interactions). We will include other test results that are not prone to biotin interference as negative controls (i.e., Abbott hs-cTnI, HbA1c and Cystatin C).

5. Main Hypothesis/Study Questions:

**Question 1:** Prevalence of high dose biotin supplement use (i.e., ≥5 mg daily and ≥10 mg daily) in the ARIC Visit 6.

**Hypothesis 1:** Biotin supplement use in large doses (i.e., ≥5 mg daily and ≥10 mg daily), compared to those with no or low dose biotin supplement use (i.e., multivitamin uses), will be associated with biomarker concentrations for the following biotinylated assays (cardiac Troponin T and NT-proBNP) and non-biotinylated assays (Abbott hs-cTnI, Tosoh HbA1c, Gentian Cystatin C, included as negative controls):

a) levels of cardiac Troponin T or NT-proBNP will be lower in biotin users of large doses compared to no or low biotin supplement use (visit 6)

b) levels of hs-cTnI, HbA1c, Cystain C will be comparable between biotin users of large doses compared to no or low biotin supplement use (visit 6)

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**

This study will examine cross-sectional associations of biotin supplement use and 2 biotinylated assay results cardiac Troponin T and NT-proBNP at the ARIC Visit 6.

**Inclusion/Exclusion**

a) ARIC Visit 6 participants with biotin supplement use information available at Visit 6 from the Medication Survey

We will examine the overlap of biotin supplement only use with multivitamin use. We may restrict our analyses to multivitamin users since most biotin exposure is likely to come from multivitamins.

**Exposures**

ARIC Visit 6 participants completed Medication Surveys and brought all the medications they used in the past two weeks or their containers. The information was then recorded by trained personnel during the visit. In addition to medications, supplement use information was also recorded during the survey.
Biotin supplementation can come from single biotin and/or multivitamins. Single biotin supplement usually comes in large doses. However, biotin dose in multivitamins are low (e.g., less than 1 mg) and unlikely to cause biotin interference with lab tests, except those multivitamins specifically marketed for hair, nails, and skin improvement, which can contain large doses of biotin. Therefore, we will use the following approach to extract large dose biotin supplement use information.

a). Information on single biotin supplement use and dose will be extracted from the Medication Surveys conducted in ARIC Visit 6 (a preliminary examination showed 97 and 21 of the Visit 6 participants who completed the Medication Surveys (~3800) were indicated single biotin supplement use of ≥5 mg daily and ≥10 mg daily, respectively)
b). Information on use of multivitamins specifically marketed for hair, nails, and skin improvement will be extracted from the Medications Surveys. Biotin dose information from these special "multivitamins" will be estimated by information searched online (a preliminary examination showed 587 of the Visit 6 participants who completed the Medication Surveys (~3800) were indicative of multivitamins use; 9 and 6 of them were indicated single biotin supplement use of ≥5 mg daily and ≥10 mg daily, respectively).

Sensitivity analyses will be performed to determine if results remain the same when excluding the biotin users with amended dose information (e.g., multivitamins specifically marketed for hair, nails, and skin improvement).

**Outcomes**

a) Cardiac Troponin T and NT-proBNP test results at Visit 6
b) Other test results included in Visit 6 that are not prone to biotin interference will be used as negative controls (i.e., Abbott hs-cTnI, HbA1c and Cystatin C)

**Other Variables**

Other variables that are known to affect or associated with cardiac Troponin T and NT-proBNP (e.g., health status of participants) at the Visit 6 will be considered, if such information is available in ARIC. These include use of multivitamins, age, sex, race-center, hypertension, diabetes, coronary heart disease, heart failure, chronic kidney disease, and other important cardiovascular risk factors (e.g., high LDL-cholesterol, high hemoglobin A1c, smoking, being overweight or obese) at Visit 6. We will also consider numerous other variables which may be associated with both likelihood of taking biotin, such as factors associated with SES and BMI at the Visit 6.

**Data analysis**

For laboratory analyses, continuous biomarkers will be assessed for normality and we will log-transform biomarker concentrations with right-skewed distributions. Daily supplemental biotin intake will be categorized as no use, 0-1 mg (e.g., multivitamin uses excluding skin, nails, hair), ≥ 5 mg, ≥10 mg. Linear regression will be used to obtain mean biomarker concentrations for each supplemental biotin intake category adjusting for use of multivitamins, age, sex, race-center, hypertension, diabetes, coronary heart disease, heart failure, chronic kidney disease, high LDL-cholesterol, high hemoglobin A1c, smoking, being overweight or obese (BMI), and SES.
As noted above, we will also conduct analyses restricting to multivitamin supplement users, and including only individuals with single biotin supplement use information.

**Anticipated methodologic limitations and challenges**

Confounding by indication is the major challenge that this study will likely face. We have included a list of confounding variables (use of multivitamins, age, sex, race-center, hypertension, diabetes, coronary heart disease, heart failure, chronic kidney disease, high LDL-cholesterol, high hemoglobin A1c, smoking, being overweight or obese [BMI], and SES). Furthermore, we have included non-biotinylated test results (XX and XX) as negative controls.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes ____ 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 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? ____ 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/mantrack/maintain/search/dtSearch.html](http://www.cscc.unc.edu/aric/mantrack/maintain/search/dtSearch.html)

__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)?

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* __2009.16 _______)

___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* __________ __________ __________)
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