

[Peijin Han]

ARIC Manuscript Proposal #2810

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Priority: _____

1.a. Full Title: Is dietary choline intake associated with risk of lethal prostate cancer in ARIC?

b. Abbreviated Title (Length 26 characters): Choline and Prostate Cancer

2. Writing Group:

Writing group members: Peijin Han, Elizabeth A Platz, Corinne Joshu, Donald Coffey, Aurelian Bidulescu, Steven H. Zeisel, and other interested ARIC investigators

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: Han, Peijin

Address: Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

Phone: (410) 522-8765

Fax: (410) 614-2632

E-mail: phan5@jhu.edu

ARIC author to be contacted if there are questions about the manuscript and the first author does not respond or cannot be located (this must be an ARIC investigator).

Name: Elizabeth A Platz

Address: Department of Epidemiology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD, USA

Phone: (410) 614-9674

Fax: 410-614-2632

E-mail: eplatz1@jhu.edu

3. Timeline: The manuscript will be submitted by the end of the August 2017.

4. Rationale: Choline is an essential nutrient very pertinent to cancer studies. It is the only nutrient for which dietary deficiency causes development of hepatocarcinoma without any known carcinogen. We propose to study the association of dietary intake of choline and its metabolite betaine (trimethylglycine), which can also be consumed in the diet, with lethal

prostate cancer. Choline is a precursor of phosphatidyl choline (a component of cell membranes); is a precursor of acetylcholine (a neurotransmitter); and via betaine, is a methyl donor to homocysteine. Total choline is more abundant in prostate cancer tissue relative to normal prostate tissue,^{1,2} and is higher in higher Gleason sum disease (a poor prognostic factor) compared with lower Gleason sum disease.¹ Given its abundance, choline is used as a PET scan agent for detecting bone metastases in men with prostate cancer.³

Two prospective studies have directly investigated the association between choline and prostate cancer. The Health Professionals Follow-Up Study (HPFS) investigators observed that in men without a cancer diagnosis at baseline, risk of lethal prostate cancer (incident diagnosis of metastatic prostate cancer, progression to metastatic disease, or death from prostate cancer) increased across quintiles of intake of choline, which was primarily from the consumption of eggs, red meat, and milk.⁴ After multivariable adjustment, risk of lethal prostate cancer was 70% higher comparing the top versus the bottom quintile. In men in the HPFS with prostate cancer that was not metastatic at diagnosis, risk of progression to metastasis or death was higher in the top quintiles compared to the bottom quintile. Betaine intake was not associated with either outcome in the HPFS. The other prospective study, a nested case-control study in Sweden, found that higher blood concentration of choline was associated with an increased risk of prostate cancer; about 25% of the cases were considered to be high-risk disease.⁵ Other studies have focused on the association between the source of choline (e.g., egg, red meat, poultry) and prostate cancer incidence and survival. For example, egg consumption, which is one of the top contributors to choline intake (including in ARIC⁶) was associated with increased risk of lethal prostate cancer among healthy men,⁷ and in men with early stage prostate cancer, greater consumption of eggs and poultry with skin was associated with 2-fold increases in risk of prostate cancer recurrence/progression.⁸ However, in the latter study, consumption of red meat, fish or skinless poultry, which are also sources of choline, was not associated with prostate cancer recurrence or progression.⁸ In a meta-analysis, a 5 egg per week increase in intake was associated with a 50% higher risk of fatal prostate cancer; no association was present for total prostate cancer.⁹

While the findings of the two prospective epidemiologic studies on choline^{4,5} are compelling, the extent of evidence for choline intake influencing the development of prostate cancer with a lethal phenotype or on the progression of prostate cancer remains limited. Thus, we will evaluate the association of choline and betaine intake with risk of lethal prostate cancer and with case-fatality in the Atherosclerosis Risk in Communities (ARIC) Study.

We propose to address this research question in ARIC because 1) the extent of choline intake has been documented in ARIC; 2) the evaluation of choline and betaine intake in relation to another chronic disease has been documented to be feasible in ARIC; and 3) we expect the findings to complement those from the HPFS. With respect to 1), in ARIC, the median choline intake in white and African-American men is 304 and 295 mg/day, respectively¹⁰ (in the HPFS, the median is higher, 385 mg/day⁴). With respect to 2), choline and choline plus betaine intakes were not inversely associated with risk of incidence coronary heart disease as had been hypothesized and the results remained similar after measurement error correction.⁶ With respect to 3) ARIC has a different composition of participants than in the HPFS. Most HPFS men are white and are highly educated (all have a graduate degree). In contrast, about 25% of the men in ARIC are African-American and their socioeconomic and educational backgrounds are much more diverse. African-American men have higher prostate cancer incidence and mortality rates and are more likely to be diagnosed with an advanced disease stage than white men.^{11,12}

This study team has previously documented the ability to study African-American-white differences in lethal prostate cancer risk factors in ARIC, as we propose here, and found that some risk factors are similar in African-American and white men (e.g., smoking is positively associated, Jones MR et al. in process) and others differ (e.g., obesity is a positively associated in white, but not associated in African-American men, Joshi CE et al. in process).

Main Hypothesis/Study Questions:

Study Question 1: Determine whether dietary choline and betaine intake are associated with risk of total, lethal, and fatal prostate cancer in men without prostate cancer at baseline.

We hypothesize that higher dietary intake of choline, but not betaine (as in the HPFS study), is associated with an increased risk of lethal and fatal prostate cancer, but not total prostate cancer in men without prostate cancer at baseline.

Study Question 2: Determine whether dietary choline and betaine intake are associated with risk of death from prostate cancer in men diagnosed with prostate cancer during follow-up.

We hypothesize that higher intake of dietary choline, but not betaine (as in the HPFS study), is associated with increased case-fatality in men with prostate cancer after taking into account prognostic factors.

Study Question 3: Determine whether the associations of choline and betaine with these outcomes differ between white and African-American men.

We hypothesize that the associations of choline and betaine with these prostate cancer outcomes do not differ between white and African-American men when comparing the same ranges of intakes.

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:

Q1: We will conduct a prospective cohort study of eligible male participants in ARIC. We will begin follow-up at entry into ARIC. Follow-up will end at the outcome date (prostate cancer diagnosis for total or lethal; date of prostate cancer death for fatal), death from another cause, study withdrawal, or 2012 (the last cancer file) whichever comes first.

Q2: We will conduct a prospective cohort study of eligible male participants in ARIC who were diagnosed with prostate cancer since their enrollment in ARIC. We will begin follow-up at the date of prostate cancer diagnosis. Follow-up will end at the date of prostate cancer death, death from another cause, study withdrawal, or 2013 (the last death file), whichever comes first.

Q3: We will use the two analytic cohorts from Q1 and Q2 and will stratify by race (African-American or white).

Inclusion/exclusion criteria:

Q1: We will include only men without a diagnosis of cancer at baseline. We will exclude men who did not consent to studies on other diseases such as cancer and who did not sufficiently complete the FFQ (men missing ≥ 10 responses to food item questions) or who had extreme energy intake (men ≤ 600 or $\geq 4,200$ kcal) as done previously in ARIC.¹³

Q2: Same criteria as Q1, but restricted to men who were diagnosed with prostate cancer after baseline. We will exclude men without confirmed prostate cancer, and men with prostate cancer for whom insufficient information is available on stage at diagnosis.

Q3: Same criteria as Q1 or Q2, but restricted to only African-American or white men.

Outcome:

Q1, Q3: Incident total prostate cancer (first primary), lethal prostate cancer (first primary metastatic at diagnosis or died of a previously diagnosed first primary prostate cancer), and fatal prostate cancer (died of prostate cancer irrespective of whether a first primary) in men without a diagnosis of prostate cancer at baseline.

Q2, Q3: Death from prostate cancer in men diagnosed with prostate cancer after baseline (case-fatality).

Exposure:

Intakes of dietary choline and betaine were quantified, during the baseline visit (1987–1989) and visit 3 (1991-1993), with a 66-item version of the Willett semi-quantitative food frequency questionnaire (FFQ). Intakes of choline and betaine were estimated as the sum of daily intakes, using U.S. Department of Agriculture (USDA) choline and betaine content in common foods database. Choline and betaine intake will be energy adjusted using the residual nutrient model.¹⁴ Like done in a prior ARIC analysis,⁶ we will also create an exposure variable for the sum of choline plus betaine, given that betaine is a metabolite of choline but also can be consumed in the diet.

Covariates:

Q1: Model 1: Race by ARIC field centers, education; Model 2: Model 1 + updated baseline body mass index (BMI), height, updated smoking status, updated diabetes status (physician diagnosis or use diabetes medications), and updated statin drug use (lethal prostate cancer risk factors). Model 3: Model 1 + Model 2 + intake of folate, methionine, and vitamins B6 and B12 (components of the one carbon metabolism pathway, which includes choline and betaine).

Q2: Same as Q1 plus adjustment for stage and Gleason sum and for the time between the date of FFQ completion and date of diagnosis in Models 1 – 3.

Q3: Same as Q1, but exclude race by ARIC study centers.

Analysis plan:

Q1: Cox proportional hazards regression will be used to calculate the hazard ratio (HR) of total, lethal, and fatal prostate cancer. The time metric will be participants' age. Men will contribute person-time at risk until the outcome of interest, death due to other causes, or last known follow-up. We will categorize the intake of choline, betaine, and their sum into quintiles and adjust choline and betaine intake by total energy using the residual method.¹⁴ We will model intakes in three ways: 1) Baseline method: using choline and betaine in baseline visit as exposure; 2) Updated method: using the updated choline and betaine intake as exposure (2 time points); 3) Cumulative updated method: using the average choline and betaine intake of baseline and updated as exposure. We will adjust for covariates as follows: Model 1: Race by ARIC field centers, education; Model 2: Model 1 + updated baseline body mass index (BMI), height, updated smoking status, updated diabetes status (physician diagnosis or use diabetes medications), and updated statin drug use (lethal prostate cancer risk factors). Model 3: Model 1 + Model 2 + intake (continuous variables) of folate, methionine, and vitamins B6 and B12 (components of the one carbon metabolism pathway, which includes choline and betaine). We will test for trend by entering into the model a single ordinal term with values equal to the median of each quintile of choline, betaine, or their sum.

Because of the inter-relatedness of choline, betaine, and other components of the one-carbon metabolism pathway (e.g., when folate is low, choline demand increases; when betaine is low, folate demand increases, choline demand (as discussed previously in Bidulescu A et al.⁶), using Model 2, we will also determine whether folate, methionine, or vitamins B6 and B12 modify the association of choline, betaine, and their sum with risk of total, lethal, and fatal prostate cancer. We will stratify the analysis by the median (or 25th percentile, if feasible to reflect “low” intake) of folate, methionine, and vitamins B6 and B12, and assess stratum-specific differences in the HRs. To test for interaction, we will enter into Model 2 main effects terms for folate, methionine, or vitamins B6 and B12 (continuous) along with a cross-product term for choline, betaine, and their sum (continuous) with these. The statistical significance of the interaction will be evaluated by the likelihood ratio test.

Statistical analyses will be conducted using Stata 14.0 software. Statistical tests will be 2-sided and $p < 0.05$ will be considered to be statistically significant.

Q2: A similar approach will be used except that the time scale will be time since diagnosis and all models will be adjusted for the prognostic factors stage and Gleason sum, and for time between the date of FFQ completion and the date of diagnosis

Q3: Same as Q1, but restricted to only African-American or to white men. To test for interaction, we will enter into Model 2 main effects term for race along with a cross-product term for race

and choline, betaine, and their sum (continuous). The statistical significance of the interaction will be evaluated by the likelihood ratio test.

Methodologic challenges: The primary challenges are nondifferential measurement error of choline and betaine intake, and small sample size for key outcomes.

1. For Q1: In ARIC, an FFQ was administered only on 2 occasions, the baseline visit (1987–1989) and visit 3 (1991-1993). Whether early follow-up intake reflects more recent intake as the men age and as the US food environment changes over time is unknown. Typically, the possible effect of this measurement error is to attenuate any association for choline and betaine to the null. A prior study conducted among a subset of ARIC participants documented a reliability of 0.5 over 3 years for both choline and the sum of choline and betaine intake.¹⁰ To identify whether this error exists, we will stratify the main analysis at the median calendar year of diagnosis (time since FFQ completion) and look for diminishing HRs with time. To address this challenge, we could perform measurement error correction as was done previously.⁶ However, Richman et al.⁴ found that after applying a 16-20 year lag for exposure, men in the fifth quintile of choline intake had a risk of lethal prostate cancer > 3-fold that of men in the lowest quintile, while men in the highest quintile of choline intake had a 70% increased risk of lethal prostate cancer compared with men in the lowest quintile if no lag time was applied. Thus, it is possible that use of baseline and visit 3 intake may better classify the men with respect to etiologically relevant intake than more recent intake.
2. For Q2: The time between pre-diagnostic FFQ completion date and date of prostate cancer diagnosis varies from man to man. We will adjust for the variability in this time using indicator variables. We will also confirm that the association does not differ by median time between FFQ completion and date of diagnosis via stratified analysis. We will not be able to study post-diagnostic intake because the majority of cases were diagnosed after visit 4 and the 2 FFQs are before then.
3. For Q1-Q3: If we observe possible associations but that are not statistically significant we will propose studying these same associations within a consortium of cohorts with FFQ data.

7.a. Will the data be used for non-CVD analysis in this manuscript? 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? 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 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.csc.unc.edu/ARIC/search.php>

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)?

MP1049 - Dietary choline intake as a predictor of occlusive coronary events. First author: Aurelian Bidulescu, Senior author: Gerardo Heiss

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

11.b. If yes, is the proposal

- A. primarily the result of an ancillary study (list number* 2011.07, 1995.04)
 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.

13. Per Data Use Agreement Addendum, approved manuscripts using CMS data shall be submitted by the Coordinating Center to CMS for informational purposes prior to publication. Approved manuscripts should be sent to Pingping Wu at CC, at pingping_wu@unc.edu. I will be using CMS data in my manuscript Yes No.

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