ARIC Manuscript Proposal # 1049

PC Reviewed: _11/04/04	**Status: _A___**	**Priority: __2__**
SC Reviewed: __11/04/04	**Status: _A___**	**Priority: __2__**

1.a. Full Title:

Dietary choline intake as a predictor of occlusive coronary events.

b. Abbreviated Title (Length 26 characters): dietary choline and risk of CHD events.

2. Writing Group (list individual with lead responsibility first):

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3. Timeline:

We plan to submit an abstract to the AHA Annual Conference for 2006 (deadline, October 2005). It is anticipated that a draft of a manuscript would be completed by the end of Fall 2005.

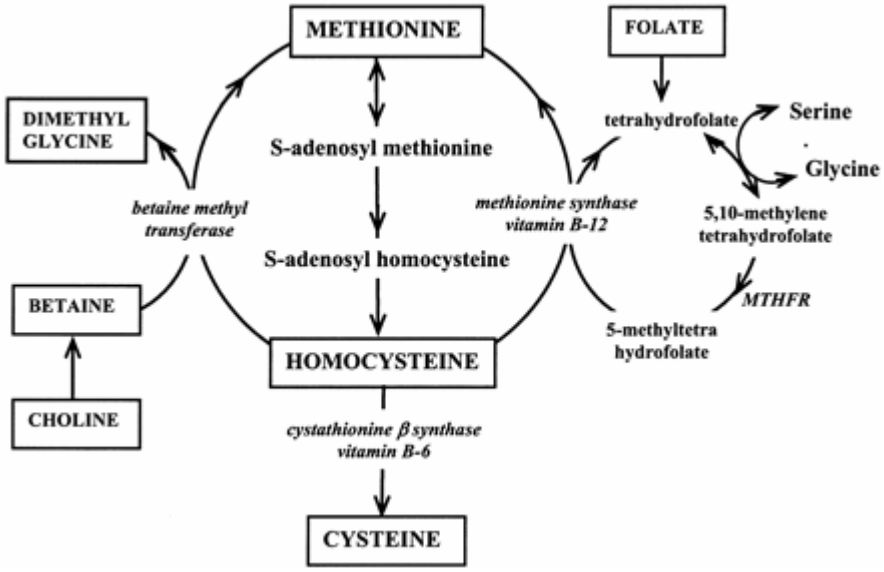
4. Background and Rationale:

Myocardial infarction often occurs among persons without traditional risk factors (reviewed in 1). The 1996 Bethesda Conference acknowledged elevated blood levels of homocysteine as a possible new cardiac risk factor (reviewed in 2). A series of recent meta-analyses suggests that elevated blood level of homocysteine is an independent risk factor for occlusive vascular events, with and without taking in consideration the interaction potential of methylenetetrahydrofolate (MTHFR) genotype. A meta-analysis by Ueland et al. (3), using 14 prospective studies including the ARIC study, yielded a pooled odds ratio (OR) of 1.20 (95% C.I. of 1.14-1.25). In the ARIC study (4), the OR (with the 95% C.I.) was 1.15 (0.68-1.92). These odds ratio estimates are lower compared with the pooled OR of another meta-analysis (5), using mainly case-control studies. In this study the odds ratio for coronary artery disease of a 5 μmol/L homocysteine increment was 1.6 (95% CI, 1.4 to 1.7) for men and 1.8 (95% CI, 1.3 to 1.9) for women. In another meta-analysis (6), a 25% lower homocysteine level was associated with an 11% (OR, 0.89; 95% CI, 0.89-0.96) lower ischemic heart disease risk.

The experimental studies that took into consideration the mechanisms by which homocysteine exerts its toxicity showed that this compound acts in several ways (reviewed in 7). Homocysteine has a direct cytotoxicity on vascular endothelium. It produces peroxydation of

low-density lipoproteins (LDL), and diminishes the production of nitrogen oxide (NO). It has pro-coagulant activities by acting on platelets and on several coagulability factors. Treatment of cultured endothelial cells with 0.5-10 mM homocysteine increased Factor V activity and prothrombin activation by Factor Xa (8). There is also an association between exogenously applied homocysteine and impaired activation of the natural anticoagulant protein C (9). In a study on patients with peripheral arterial disease (10), it was shown that plasma concentration of Von Willebrand factor was decreased in subjects with elevated levels of plasma homocysteine treated with pyridoxine plus folic acid. Since then, a series of similar studies, also involving protein C, have been conducted, and have shown similar results. Therefore, there seems to be a relationship between the above-mentioned coagulability factors and plasma levels of homocysteine, and, hence, supposedly, dietary betaine.

Homocysteine is a sulfur aminoacid whose metabolism stands at the intersection of two pathways. One of them is remethylation to methionine, which requires folate and vitamin B_{12}. In an alternative reaction, betaine, a choline derivative, serves as a donor of methyl groups to homocysteine to form methionine (11). Folate and choline are metabolically interrelated (idem). When folate availability diminishes there is an increased demand for choline as a methyl donor. When choline availability is decreased, the demand for folate methyl groups is increased (12).



In an experimental choline depletion/repletion study (13) it was found that feeding healthy men a choline deficient diet with adequate methionine and folate for 3 weeks resulted in low plasma choline and phosphatidylcholine and liver dysfunction, all of which were reversed upon choline repletion. The authors concluded that choline is an essential nutrient for humans when sufficient methionine and folate are not available in the diet. At present, the U.S. Institute of Medicine's Food and Nutrition Board established choline as an essential nutrient for humans (14). Foods that are especially rich in choline compounds are milk, liver, eggs and peanuts.

Recently, a betaine supplementation trial with doses in the range of habitual dietary intake on fasting and post-methionine loading plasma homocysteine concentrations in healthy adults (15) showed that supplementation of betaine at doses as low as 1.5 g/day lowers plasma homocysteine concentration in healthy adults. It has been shown that not only betaine supplementation lowers mildly elevated plasma homocysteine (16) but there seems to be an apparent dose-response relationship between betaine supplementation, at doses in the range of dietary intake, and plasma homocysteine concentrations (15). Meanwhile new studies show that,

in a population possibly deficient in serum choline (betaine), supplementation with folate (vitamins B) may not be efficient in reducing blood homocysteine levels (17,18). Because folate and choline methyl donation metabolic pathways can be interchangeable, it has been suggested that both folate and choline be considered in epidemiological studies assessing the relationship between dietary intake of these compounds and cardiovascular diseases. Thus far, epidemiological studies assessing the relationship between intake of folate and/or vitamin B_6, B_{12} and CVD outcomes do not include dietary choline (betaine) intake as a covariate, because data were not available on the choline concentration of common foods. Nevertheless, choline or betaine treatment has been used to lower high plasma homocysteine concentrations (19, 20). It is now possible to conduct studies for normal range values because the concentrations of choline, betaine and choline-containing compounds in common foods have been characterized to some degree (21,22).

The content of betaine in wine and the high average consumption of wine in France has been proposed as one possible explanation of the "French paradox", namely the lower than predicted coronary heart disease mortality in that country (23). In the U.S., dietary intake of betaine is estimated at 0.5-2 g/day. The main food sources of betaine are spinach, beets and wheat products (21).

Very recently, periconceptional dietary intake of choline and betaine in relationship with neural tube defects in offspring was assessed by a group of researchers from California (24). They investigated, in a case-control setting, whether maternal periconceptional dietary intake of choline and its metabolite betaine influenced neural tube defects (NTD) risk. Dietary intakes of choline were associated with reduced NTD risks. Controlling for intake of supplemental folic acid, dietary folate, dietary methionine, and other covariates did not substantially influence risk estimated for choline.

If elevated blood level of homocysteine (including related compounds) is a risk factor this could and must be borne out by future research due to its modifiable nature. It is estimated that almost half of US women have low intakes of folate, a nutrient that reduces (by metabolic methylation) the blood levels of homocysteine. Populations vulnerable to choline deficiency exist, including growing infants, pregnant or lactating women and patients fed by total parenteral nutrition (25). Therefore, providing evidence for a correlation between choline intake and CHD risk would have practical implications for the public health arena. This could represent a starting point for dietary strategy for prevention and/or food fortification especially for the segments of the population mentioned above.

Considering that only a recent case-control study of the dietary intake of choline has been reported, this proposed study represents the first to address this issue in a cohort setting. The participation and support of the UNC Department of Nutrition represents one of the keys for the success of this proposed study. Also, access to an ongoing cohort such as the ARIC study will introduce considerable cost efficiencies.

5. Main Hypothesis/Study Questions:

We will test the hypothesis that dietary total choline intake (including betaine, its oxidized form) - as established from the semi-quantitative Food Frequency Questionnaire (FFQ) used in ARIC - is inversely related to the risk of coronary occlusive events. We will take into account the potential interaction between choline, folate, and vitamins B (B_6 and B_{12}), respectively.

Another hypothesis tested will be that Von Wilebrand factor level (one of the coagulability factors measured in ARIC participants) is directly related to the risk of incident CHD, by level of dietary choline intake, adjusting by blood folate level.

6. Data (variables, time window, source, inclusions/exclusions):

The following variables will be needed for the analysis. Regarding the outcome, in ARIC there are more than 650 incident CHD events, up to the year 2001 – the variable INC_BY01, in inc_by01.sd2 events dataset. Concerning confounding factors, the regression models will be adjusted for age, gender (8,710 women), race (4,266 blacks), ARIC center, smoking (status, 4,132 active smokers, and pack-years), drinking (8,768 current drinkers), BMI, education level (3,767 with less than high school) as a measure of SES, diabetes (1,870 participants), and hypertension (5,504 study subjects). In terms of the second hypothesis, Von Wilebrand factor level, the dataset hema.sd2 from Visit 1 will be used for the analysis. In this dataset, the variable HEMA17, the Von Wilebrand value, was measured in 15,519 participants. We will use SAS in our analysis.

Cox proportional hazards regression models will be used to calculate the multivariate adjusted risk ratio (a HR) of incident CHD in relation to tertiles of dietary total choline and betaine. Several models for adjustment will be created. Dietary choline intake will be computed by multiplying the choline content of each food item by the frequency of its daily consumption and summing over all items. Also Cox proportional hazards regression models will be used to calculate the multivariate adjusted hazard ratio of incident CHD in relation to tertiles of Von Willebrand factor, assessing dietary total choline and folate as possible effect modifiers/ confounders. To evaluate the potential effect-measure modification of folate and vitamins B (Vitamin B_6 and Vitamin B_{12}) for the dietary choline-CHD association, a test for interaction (homogeneity) will be used.

In the second part of the proposed project, a random error measurement component, we will assess the reliability of the dietary instrument by ethnicity and gender, in a random sample of 443 subjects whose dietary intake was measured during the second visit of ARIC, within each geographic center. This will be contrasted with the dietary information collected in Visit 1 for the same participants. Nested random effects models will be used to estimate the between-persons variance, and within-person variance. With these estimates, we will calculate a reliability coefficient, as the proportion of total variance attributable to the between-person component. This coefficient will be used to calculate a weighted dietary choline intake.

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 ICTDER02 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 ICTDER02 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 ICTDER02 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://bios.unc.edu/units/cscc/ARIC/stdy/studymem.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)?**

    NA

11. **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.**

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