ARIC Manuscript Proposal # 1010

PC Reviewed: 05/06/04 **Status: A** **Priority: 2**
SC Reviewed: 05/07/07 **Status: A** **Priority: 2**

1.a. Full Title: Omega-3 fatty acids, hypertension and risk of cognitive decline among older adults: The Atherosclerosis Risk in Communities (ARIC) study

b. Abbreviated Title (Length 26 characters): Fatty acids and cognition

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

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3. Timeline: September, 2004 – May, 2006

Data analysis: September 2004-December 2004
Literature review: January 2005-April 2005
Write-up of the manuscript(s): May 2005-September 2006
Review/editing of the manuscript(s): October 2006-December 2006
Submission to ARIC committee: January 2006
Submission to peer-reviewed journal: February-May 2006

4. Rationale:

Cognitive impairment is a major health concern that affects dependence in basic daily activities in older age and thus special attention should be focused on preventing its occurrence. Many risk factors have been studied in relation to cognitive impairment in old age, the most notable one being elevated blood pressure (Engels et. al, 1993; Palombo et. al, 1997; Kilander et. al, 1998; Swan et. al, 1998; Harrington, 2000; Knopman et. al, 2001; de Moraes et. al, 2002; Elias et. al, 2003). Recent research developments indicated that omega-3 fatty acids which are mostly prominent in fish and other sea food may also be an important factor that could prevent both cognitive impairment and decline. However, so far, evidence has been confined to biochemical studies (Conquer et. al, 2000; Tulley et. al, 2003; Heude et. al, 2003) and a few cohort studies (Morris et. al, 2003; Kalmijn et. al, 1997; Kalmijn et. al, 2004) . Most of the findings, although not conclusive, leaned towards a protective effect of omega-3 fatty acid supplementation in the diet. Experimental animal studies suggest a plausible pathway by which hypertension and low dietary omega-3 fatty acid intake may interact in increasing the risk of cognitive impairment and decline (Edmond, 2001; Moore, 2001; Yamagata et. al, 1997; Tang et. al, 1993). The present study will assess the independent effects of low omega-3 fatty acids (both in the diet and in plasma) on cognitive decline as well as the interaction of this risk factor with elevated blood

pressure. The results of this study may have great public health and biomedical implications. First, it may suggest a combined benefit of supplementing diet with omega-3 fatty acids and preventing hypertension in middle-age adults and second, it may improve outcomes of drug development and clinical trials that have cognitive impairment as a side effect. For this purpose, we will be using baseline and visit 2 exposure data from the Atherosclerosis Risk in Communities (ARIC) study and follow-up period will span visits 2 (1990-92) through visit 4 (1996-98) to determine the extent of cognitive decline.

5. Main Hypothesis/Study Questions:
The main hypothesis to be tested is that low omega-3 fatty acid status enhances cognitive decline among older adults and that hypertension has a synergistic effect in that association.

The specific study questions are:
1. Is low *dietary* consumption of omega-3 fatty acids vs. other poly-unsaturated fatty acids (PUFA) at baseline (i.e. visit 1) related to incidence of cognitive decline among older adults between visits 2 and 4?
2. Is low *plasma* omega-3 fatty acids vs. other PUFA at baseline (i.e. visit 1) related to cognitive decline among older adults between visits 2 and 4?
3. Do these two risk factors interact with hypertension to increase the risk of cognitive decline? In other words, is hypertension an effect modifier in the relationships that are investigated in questions 1. and 2.?

If the results warrant that more than one manuscript be submitted for publication the author will notify the ARIC Publications Committee, and each manuscript will be submitted for review by the ARIC steering committee prior to sending it to a journal.

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

6.1. Outcome variable:
Three measures of cognitive functioning were made for visits 2 and 4 of the ARIC study, and these measures relied on Delayed Word Recall (DWR), Digit Symbol Substitution portion of the Revised Weschler Adult Intelligence Scale (DSS/WAIS-R) and Word Fluency (WF) screening tests (http://www.cscc.unc.edu/aric/). Each of these test obey certain rules of administration, which will be summarized in the next sub-section.

6.1.1. Delayed Word Recall (DWR): This screening test is given first by asking the subject to read aloud 10 words and use them in a sentence the first time and then read them again and use them a second time in a sentence. Then, after the participant completes the following test (DSS/WAIS-R) which takes about 5 minutes, he is asked to recall the 10 words within a 60 second time limit for each word. The ten words used in ARIC were: chimney, salt, harp, button, meadow, train, flower, finger, rug and book. The scoring of DWR is between **0** and **10**, and hence only the correct delayed recall of words is given a score.

6.1.2. Digit Symbol Substitution (DSS/WAIS-R): A list of 100 redundant digits are given and for each single digit (ranging between 1 and 9), a corresponding symbol must be entered. If the symbol entered is wrong it is subtracted from the perfect score of **100**.

6.1.3. Word Fluency (WF): The subject is asked to record up to 20 words using the letters F, A and S and to construct these words, the subject is given only 60 seconds per letter. The

total score corresponds to the total number of words written in the three columns (each column corresponding to one letter). Therefore, the maximum score is **60**.

The DWR, WF, and DSS/WAIS-R were administered by trained interviewers and since all interviews were tape-recorded, quality checks were feasible for at least a random sample to ensure acceptable performance.

6.1.4. Composite score for cognitive decline

For the purpose of assessing cognitive decline, there are several options at hand. However, the one that we chose will attempt to create a single measure of decline that would combine all three screening tool scores together. In order to do that, the difference between visits 2 and 4 will be computed for each screening tool separately. Then, the three differences will be used to compute a common score difference, using the principal component analysis technique, which is a method to decrease the number of variables by extracting a reduced number of factors based on common variance. It is expected that all three differences will load heavily on a single factor or component. Consequently, the factor score that is estimated will rank individuals according to the degree of decline between these two visits, averaging out declines on all three instruments. This factor score is a standard z-score that has an average of zero and a Standard Deviation (SD) of 1. For our purpose, a decline which would place a person in the lower 20% of the distribution of this factor score will be considered as a significant decline. A similar approach has been adopted elsewhere (Stewart et. al, 2003; Kalmijn et. al, 2004; Logroscino, 2004). Consequently, subjects having declined by a value that is equal or greater than the absolute value of this criterion would have scored positive on the screening tools.

6.2. Main Exposures

6.2.1. Dietary omega-3 fatty acids

Usual dietary intake will be estimated from an interviewer-administered semi-quantitative questionnaire modified from a 61-item questionnaire developed and validated by Willett and colleagues (Willet, 1985). In all visits of the ARIC study examinations, the subjects were asked how often, on average, they had consumed certain foods in portions of a specified size (e.g., 85 to 113 g [3 to 4 oz] of canned tuna fish) during the preceding year. There were nine possible responses, ranging from "almost never" to "more than six times per day." Daily intake of nutrients will be calculated by multiplying the nutrient content of each food in the portion specified by the frequency of daily consumption and summing the results. The nutrient content of each food can be obtained from the Harvard nutrient data base (Willet, 1985) for which the primary source was the Department of Agriculture handbook (CFEI, 1976-89).

Fish consumption, the main dietary source of n-3 fatty acids will be estimated by summing the reported consumption of three items: 85 to 113 g of canned tuna fish, 85 to 142 g (3 to 5 oz) of dark-meat fish (e.g., salmon, mackerel, swordfish, sardines, or bluefish), and 85 to 142 g of other fish (e.g., cod, perch, or catfish). The eicosapentaenoic acid and docosahexaenoic acid content of these foods is estimated to be 190 and 500 mg, respectively, for tuna fish, 560 and 780 mg for dark-meat fish, and 240 and 460 mg for other fish (Shahar et. al, 1994). In our present study, dietary fatty acids will be analyzed from the food frequency questionnaire for visit 1.

6.2.2. Plasma omega-3 fatty acids

Fasting blood was collected according to the ARIC study wide protocol. The Minneapolis field center conducted the analysis for visit 1 blood specimens among the white segment of the study population in that center. The procedure is described in great detail by Shahar et. al (1999).

Out of the 28 fatty acids that were analyzed, the ones that are of highest interest in the present study are DHA and EPA (two omega-3 fatty acids) and AA (an omega-6 fatty acid). In addition, the ratio of total omega-3 to total omega-6 fatty acids is also of high priority. The estimated short-term reliability coefficients for DHA and EPA were, according to a study by Ma et. al (1995), around 0.5 and 0.3 respectively. In general, reliability tended to be higher for cholesteryl esters (CEs) than for phospholipids.

6.2.3. Measurement of hypertension

Hypertension can be defined based on several criteria, which include history of hypertension as told by physician, use of anti-hypertensive medication(s), or systolic (SBP) and/or diastolic (DBP) levels >=140 or >=90 mm Hg. The latter criterion is assessed by the average of the second and third of the three measurements done by a sphygmomanometer. For our purpose, the third criterion will be used as it is the most reliable and a person having high blood pressure at visits 2 or 4 or both will be labeled as hypertensive. Secondary analysis will be done to look at this effect modifier in a more subtle way, by dividing hypertensive individuals into: (1) incident hypertension: or hypertensive in visit 4 only; (2) prevalent hypertension: or hypertensive in both visits; (3) controlled hypertension: or hypertensive in visit 2 but not 4. In addition, sensitivity analysis will be conducted by including the other two criteria (namely anti-hypertensive medications or self-reported hypertension at either visit) for classifying the person as hypertensive or normotensive.

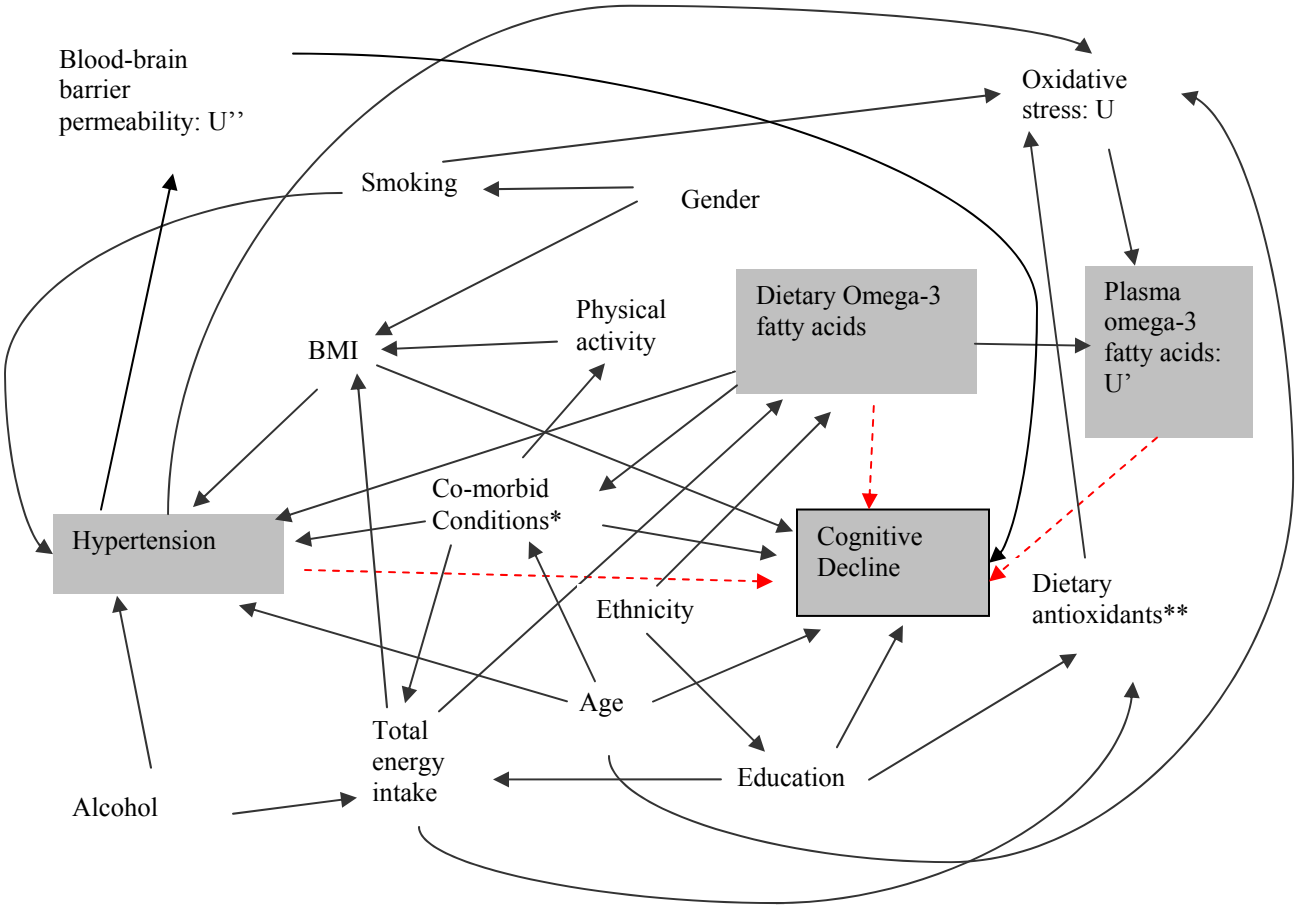
6.3. Covariates

All covariates to be included in the models as potential confounders are the ones that were measured at visit 2, with the exception of nutritional factors which will be co-measured with omega-3 fatty acids at visit 1. Hence, covariates can be subdivided into demographic, health behaviors, nutrition, and co-morbid conditions. Age (measured at visit 2), gender, ethnicity and education were all determined by interview with the respondent him/herself. Among the health behaviors, smoking will be measured on a three-level categorical scale, namely 0:"never smoked", 1:"smoked previously" and 2:"current smoker". As for alcohol use, the quantity of alcohol will be either assessed on a continuous scale or divided into 3-4 categories. Finally physical exercise will be grouped into: "sedentary", "mild/moderate", "highly active".

The nutritional factors to be taken into account include body mass index, level of antioxidants in the diet and total energy intake. Body mass index will be computed by dividing weight in kilograms of the respondent by the height-squared (in square meters). The level of antioxidants (namely Vitamins C, E and beta-carotene) will be measured from the results of the food frequency questionnaire, and the same applies to total energy intake which is estimated by multiplying food frequencies by their respective portion sizes and energy content and then

summing them over. A number of co-morbid conditions have been associated with cognitive impairment and were shown to be influenced by hypertension or poor intake of omega-3 fatty acids or a combination of the two. Some of these conditions include: type II diabetes, plasma cholesterol, triglycerides, plasma clotting factors, pulmonary function and depression. The ARIC data included self-report of diabetes as well as measurement of blood glucose, total plasma cholesterol as well as HDL and LDL components, total plasma triglycerides, plasma clotting factors, FVC and FEV1 measured by a spirometer, and depression measured by the screening tool CES-D. *Figure 1* shows a Directed Acyclic Graph (DAG) depicting the relationships between exposures, outcome and covariates. The dashed arrows represent the associations that of highest interest in this study.

Figure 1 Conceptual Framework



* These include: type II diabetes, plasma cholesterol and triglycerides, plasma clotting factors, pulmonary function and depression. The arrow going from dietary omega-3 fatty acids to "co-morbid conditions" involves an association with atherosclerosis and thrombosis, inflammation, and accumulation of beta-amyloids in the brain.
U, U' and U'': unmeasured confounders. The arrow going from Hypertension to U'' addresses the issue of hypertension disrupting transport of omega-3 fatty acids through the blood-brain barrier and thus aggravating the consequences of oxidative stress and brain damage.
** Dietary antioxidants include Vitamin C, E and beta-carotene. These act on phospholipids component of brain membranes and prevent oxidation of omega-3 fatty acids.

6.4. Inclusion/Exclusion

The ARIC cohort who was first recruited between 1987-89 included 15,792 individuals between the ages of 45 and 64 years, of which 8,985 were women. The study participants were recruited using probability area sampling. Ethnic diversity varied tremendously between Jackson county where the population chosen was exclusively African American, Forsyth county where 14% were African American and the other two communities where the population was predominantly white. The present study will focus on visits 2 and 4 which were carried out in the periods 1990-92 and 1996-98 respectively. Previous analysis carried out by de Moraes et. al (2002) shows that 11,320 individuals had complete follow-up between these two visits. However, it is important to exclude individuals that are at high risk of outcome because of a condition that is caused by hypertension, which is the main effect modifier. These conditions include history of stroke or transient ischemic attacks (TIAs) at visits 2 or 4 (n=567). In addition, subjects taking psychotropic medication which may affect their cognitive functioning at either visit were also excluded from analysis (n=2,461). In addition, 234 had missing cognitive test scores at either visit. Therefore, the final study population at risk of cognitive decline consisted of 8,058 individuals. For the present study, we will use the same exclusion criteria that were performed by de Moraes et. al (2002), but our study population will be restricted further to older adults aged 55 years or more at visit 2. Hence, it is expected that about half of the former population will be eligible for our present study, since the mean age of the 8,058 individuals was 56.7 with a SD of 5.6. There are two main reasons for restricting this population:

1) The incidence of cognitive impairment or decline is highly unlikely to occur before the age of 60 years, as shown by a wealth of previous literature.
2) The effect of hypertension on cognitive decline as shown by de Moraes et. al (2002) was much more pronounced among the population that was aged over the mean (i.e. older than 56.7 years at visit 2).

The study population selected above will be used for answering questions 1. For question 2, a subset of these subjects, namely whites selected to participate in the Minneapolis field center, will be abstracted since these are the subjects with complete data on plasma fatty acids. It is estimated that their study size would range between 900 and 1,500 depending on how balanced the allocation remains when the exclusion and inclusion criteria are applied for the first study question.

6.5. Statistical Analysis

To examine the effect of omega-3 fatty acids on cognitive decline, hierarchical logistic regression models will be conducted yielding an estimate of relative risk across quintiles of exposure. Three separate models will be run for each measure of exposure: (1) omega-3to omega-6 ratio in plasma, (2) omega-3 to omega-6 ratio in diet, (3) DHA, EPA and AA in plasma. In each of these models, the significance of an interaction term that contains exposure and effect modifier (i.e. hypertension) will be tested using the likelihood ratio test, at an alpha level of 0.05. In addition, covariates will be entered in an attempt to reduce confounding and obtain a less biased estimate of effect. These variables will be pared down to the final parsimonious model using a backward elimination process that take into the account the degree of confounding that is controlled for by adding a specific term or variable to the model. The reason for using a hierarchical model is primarily to account for intra-class correlation within clusters, since the sample was collected using a multi-stage cluster design. In this case, we will control for center-specific demographic

variables, namely mean age, gender ratio, proportion with greater than high school educational level and proportion African-American.

All analyses will be carried out using STATA version 8.0. Dummy variables will be created for categorical variables. Covariates will be kept in the model at first if they showed a moderate to strong association with cognitive decline. For the multivariate models, these variables will be kept if they were found to change the estimate of interest by more than 10%. In other words, the criterion used would be LnCoOR= $Log(OR_{reduced})/Log(OR_{full})$ >0.10. The final parsimonious models that would be presented will estimate the relative risk of cognitive decline across quintiles of omega-3 fatty acid exposure and will assess effect modification by hypertension status.

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

N/A

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)?

List of ARIC relevant published papers:

de Moraes S. A., Szklo M., Knopman D., Sato R. (2002). The relationship between temporal changes in blood pressure and changes in cognitive function: Atherosclerosis Risk in Communities (ARIC) Study. *Preventive Medicine* 33: 258-263.

Knopman D., Boland L. L., Mosley G., Howard D., Liao D., Szklo M., McGovern P., Folsom A. R. (2001). Cardiovascular risk factors and cognitive decline in middle-aged adults. *Neurology* 56: 42-48.

Ma J, Folsom AR, Eckfeldt JH, Lewis L, Chambless LE (1995). Short- and long-term repeatability of fatty acid composition of human plasma phospholipids and cholesterol esters. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Am J Clin Nutr*. 62(3):572-8.

Shahar E, Folsom AR, Wu KK, Dennis BH, Shimakawa T, Conlan MG, Davis CE, Williams OD (1993). Associations of fish intake and dietary n-3 polyunsaturated fatty acids with a hypocoagulable profile. The Atherosclerosis Risk in Communities (ARIC) Study. *Arterioscler Thromb*. 13(8):1205-12.

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Wang L, Folsom AR, Eckfeldt JH. (2003). Plasma fatty acid composition and incidence of coronary heart disease in middle aged adults: the Atherosclerosis Risk in Communities (ARIC) Study, *Nutr Metab Cardiovasc Dis* 13(5): 256-66.

List of previously approved ARIC proposals:

Wang L. et. al Plasma Fatty Acid Composition and Incidence of Coronary Heart Disease in Middle Aged Adults: The Atherosclerosis Risk in Communities (ARIC) Study, Manuscript # 890

Ma et. al Plasma FAs – Atherosclerosis, Manuscript # 179

Folsom et. al, Fatty acids & insulin. Manuscript # 179A

Ma et. al, Factor VII & Fatty Acids, Manuscript # 241

Zheng et. al , Plasma Fatty Acid Composition and 6-Year Incidence of Hypertension in Middle-Aged Adults, Manuscript # 416

Zheng et. al, Plasma Fatty Acid Composition and 6-Year Incidence of Non-Isulin Dependent Diabetes Mellitus (NIDDM) in Middle-Aged Adults. Manuscript # 417

Guallar et. al, Intake of omega-3 fatty acids and cardiovascular disease Manuscript # 555

Boland et. al, Plasma fatty acid composition and risk of coronary heart disease, Manuscript # 581

Eckfeldt et. al, When intra-individual variability (IIV) study specimens are done being analyzed for fatty acid composition (Fall 1990) data analysis can begin. Manuscript # 108

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.

List of cited and relevant references:

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