ARIC Manuscript Proposal #2470

PC Reviewed: 12/9/14	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title:
"Dietary protein consumption, silent brain infarcts and stroke. Results from the ARIC Study"

b. Abbreviated Title (Length 26 characters):
Dietary Protein, silent brain infarcts and stroke

2. Writing Group:
Bernhard Haring; Jeff Misialek; Rebecca Gottesman; Thomas Mosley; Alvaro Alonso; others welcome

I, *Bernhard Haring* (the first author), confirm that all the coauthors have given their approval for this manuscript proposal.

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3. July 2010 NHLBI workshop at Northwestern University
The first author has participated in the July2010 NHLBI workshop at Northwestern University.

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).

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4. Timeline / Analyses
Data preparation and analysis will begin upon approval, and manuscript drafting will commence once suitable analytical models are finalized.
All analyses will be done at the University of Minnesota. Initial drafts will be circulated among the writing group members.
The expected timeline is 2015-2016

5. Rationale

The effect of dietary protein on the risk of stroke has shown inconsistent results. While some authors claim that moderate dietary protein intake, particularly that from animal origin, is associated with a lower risk for stroke, other results indicate that in particular red meat intake may increase that risk.[1, 2] These inconsistencies may be explained by a previous focus on nutrients ('dietary protein type') instead of food groups, which may provide a more adequate assessment of the complexities of diet-disease associations. However, evidence derived from food group analyses or from community-based studies is sparse.[3-5] Most current data originate from study populations such as nurses, health professionals or selected Swedish or Japanese populations.[3-10] Conclusions regarding the relation of various sources of dietary protein with risk for stroke in the general population are difficult to draw.

Besides determining the risk for clinical strokes, elucidating the pathways underlying an association between dietary protein sources and incident stroke events is critical for prevention strategies. Thus, we further aim to examine the association of protein intake with incident subclinical (silent) brain infarcts (SCIs). SCIs emerge to be of key significance and independent strong predictor for later developing symptomatic stroke events.[11-13] So far not much is known on the relationship between dietary protein sources and incident SCIs.[14] We hypothesize that dietary protein type will not be associated with an increased risk for silent strokes based on clinical stroke data whereas certain food groups such as red or processed meat products will be associated with subclinical brain damage. Additionally, we aim to investigate if dietary protein intake modifies the risk for strokes in hypertensive individuals. Clinical trials suggest that substitution of carbohydrates by protein can reduce blood pressure.[15] As hypertension is a major risk factor for stroke, higher protein intake may reduce this risk. But whilst scientific evidence for the benefits of protein on blood pressure control is strong, to this point surprisingly little is known about the effect of varying protein intakes on stroke risk in hypertensive individuals.

6. Research Hypothesis

The primary objective of this study is to evaluate the relationship between total, animal, and plant-derived dietary protein, as well as individual protein-rich food groups, on the risk of clinical and subclinical strokes in a large, community-based cohort of middle-aged adults. Second, we will undertake additional analyses to investigate the effect of dietary protein intake on stroke risk in hypertensive individuals.

We hypothesize that dietary protein type (i.e. total, animal or vegetable protein) will not be associated with an increased risk for stroke. However, we anticipate that in detailed food group analyses food groups such as red or processed meat products will be associated with an increased risk for clinical and subclinical strokes.

7. Design and analysis

Study Population

The Atherosclerosis Risk in Communities Study (ARIC) is a community-based prospective cohort study of 15,792 middle-aged adults (aged 45-64 years at baseline) from four U.S. communities (Washington County, Md; Forsyth County, NC; Jackson, Miss; and suburbs of Minneapolis, Minn.).[16] For this analysis, only white and black adults will be included; blacks from the Minneapolis and Washington County field centers will be excluded due to small numbers. Furthermore, individuals with self-reported diabetes, fasting blood glucose ≥

126 mg/dL, non-fasting blood glucose ≥ 200 mg/dL or use of diabetes medication, a history of myocardial infarction, stroke, heart failure, coronary bypass surgery, angioplasty or with missing data on covariates of interest will not be included in our analyses as these conditions may lead to changes in diet and therefore the FFQ may not be a good representation of usual long-term diet. Last, participants with incomplete dietary information or with extreme calorie intake (<600 kcal or > 4200 kcal per day for men, <500 kcal or > 3600 kcal per day for women) will be excluded from further analysis. Our final sample size will be approximately 12,000 persons.

Assessment of protein intake

The ARIC study assessed protein intake using an interviewer-administered, 66-item food frequency questionnaire (FFQ) adapted from the 61-item FFQ developed by Willett et al.[17] The FFQ was administered to all subjects at visit 1 at baseline (1987–1989) and at visit 3 (1993–1995). The major contributors to protein intake included: unprocessed red meat, processed red meat, poultry, high-fat dairy, low-fat dairy, fish & seafood, eggs, nuts, and legumes. Vegetable protein intake will be defined as the difference of total and animal protein intake.

For assessing dietary behaviour, participants will be divided into quintiles of cumulative average intake of various protein sources. Cumulative updating of the FFQ (i.e. visit 1 FFQ for follow-up between visit 1 and visit 3 and the average of visits 1 and 3 FFQ afterwards for those who attended both examinations, or visit 1 FFQ for those who did not attend visit 3) will be used to reduce within-person variation and best represent long-term dietary behavior [18].

Assessment of stroke

The primary endpoint for this study will be stroke (definite or probable ischemic or hemorrhagic) after the completion of the first FFQ (between 1987 and 1989). The ARIC study identified incident stroke cases through hospital discharge codes and stroke deaths as previously described.[19-21]

Brain MRI

The ARIC study and MRI protocol have been described previously.[16, 22] In brief, a subset of ARIC study cohort participants were invited for a brain MRI during visit 3 (1993-1995) and in 2004 to 2006. A total of 1934 participants underwent cerebral MRI (59.7% women and 49.6% blacks). Subclinical cerebral infarcts were defined as focal, nonmass lesions ≥ 3mm that were bright on T2 and proton density and dark on T1 images. For this analysis, we will examine silent stroke incidence in participants without a history of SCIs or clinical stroke at visit 3 who developed SCIs by 2004 to 2006; thus, we will exclude individuals with a history of stroke or SCIs at baseline (visit 3).

Covariates

Height, weight, and waist circumference were measured following a standardized protocol. [16, 23] ARIC participants underwent fasting venipuncture at each examination [16]. Data on current smoking, ethanol intake, education, intake of antihypertensive or lipid lowering medication were derived from standardized questionnaires.[16] Sports-related physical activity and leisure related physical activity were assessed with the use of Baecke's questionnaire and scoring systems.[24] Diabetes will be defined as current use of glucose-lowering medications, fasting blood glucose ≥ 126 mg/dL, non-fasting blood glucose ≥ 200

mg/dL or self-reported history of diabetes. Hypertension will be defined as the average of the last two of three blood-pressure readings at the first visit (using 140mmHg or higher for systolic and 90mmHg or higher for diastolic as cut-off points).

Statistical Analysis

Characteristics of our study population will be shown by quintiles of total protein intake (Table 1). To assess the association of total, animal and vegetal protein intake with stroke incidence [total (i.e. hemorrhagic and ischemic)] (Table 2-5), we will calculate incidence rates (IR) of stroke events per 1000 person-years as the number of diagnosed cases of stroke (total, hemorrhagic or ischemic) occurring during the entire follow-up period divided by person-years of follow-up. Person-years of follow up will be defined as time from the baseline examination to the date of the first stroke event, death, lost to follow-up, or December 31, 2011. Thereafter, corresponding rate ratios will be calculated by dividing the rate among participants in each specific intake quintile by the rate among participants in the lowest quintile of intake (reference). Cox proportional hazards regression models will be used to account for potential confounding. An initial model will adjust for age, race, sex, ARIC study center, and total energy intake (minimally adjusted model). A second model will additionally adjust for smoking (current, former, never), pack years of smoking, education (less than high school, high school, more than high school), systolic blood pressure (mmHg), use of antihypertensive medication, HDLc (mmol/l), total cholesterol (mmol/l), use of lipid lowering medication, body mass index (kg/m^2), waist-to-hip ratio, alcohol intake (g/week), Baecke's physical activity score, leisure-related physical activity, carbohydrate intake (quintiles), fiber intake (quintiles), and magnesium intake (quintiles) (fully adjusted model). Median protein intake of each quintile (g/d) will be modeled as a continuous variable to test for linear trend.

The association between protein intake and incident subclinical infarcts will be assessed with a logistic regression model, adjusting for similar sets of variables as in the incident stroke analysis.

Finally, additional Cox proportional hazards models will stratify by hypertension status to examine the interaction of hypertension with protein intake for the incidence of clinical and subclinical stroke. These analyses will be based on the fully adjusted models.

8. Conclusion

We anticipate that dietary protein type will not increase the risk for clinical strokes or SCIs whereas certain food groups such as red meat and processed meat products will be associated with an increased risk for clinical and subclinical strokes.

9.a. Will the data be used for non-CVD analysis in this manuscript? No

9.b. NA

10.a. Will the DNA data be used in this manuscript? No

10.b. NA

11.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. Yes

12. 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)?

MS# 1916: Dietary predictors of structural brain MRI abnormalities

13. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ARIC Brain MRI

14a. 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.
Yes, the lead author is aware that manuscript preparation is expected to be completed in 1-3 years, and if this expectation is not met, the manuscript proposal will expire.

14.b. 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.
Yes, the lead author is aware of the Public Access Policy and that manuscript has to be uploaded to Pubmed Central.

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Tables

Table 1. Unadjusted baseline characteristics according to quintiles of total protein intake, ARIC 1987-1989

	Q 1 (low)	Q 2	Q 3	Q 4	Q5 (high)	p-trend[a]
N						
Protein intake, g/day (SD)						
Protein intake, % of total energy						
Age, years (SD)						
Women, %						
Black, %						
High school graduate, %						
Current smoker, %						
Hypertension, %						
Body Mass Index, kg/m^2 (SD)						
Waist-to-hip ratio (SD)						
Baecke Sport Activity Score (SD)						
Baecke Leisure Index (SD)						
Systolic blood pressure, mmHg (SD)						
Serum HDL, mmol/L (SD)						
Serum cholesterol, mmol/L (SD)						
Use of antihypertensive medication, %						
Use of lipid lowering medication, %						
Carbohydrate intake, g/day (SD)						
Carbohydrate intake, % of total energy (SD)						
Fiber intake, g/day (SD)						
Magnesium intake, mg/day (SD)						
Alcohol intake, g/week (SD)						
Total energy intake, kcal/day (SD)						
Total fat Intake (g/d) (Median ±SD)						
Total fat intake, % of total energy (SD)						

Values are % for categorical variables and mean (SD) for continuous variables.

[a]p-values from general linear models for continuous variables and Mantel-Haenszel 1-degree of freedom chi-square statistic

Table 2. Association of total, animal and vegetal protein intake with total stroke incidence (ischemic and hemorrhagic), ARIC 1987-2011

	Q 1	Q 2	Q 3	Q 4	Q 5	p-trend
Total Protein Intake						
Events, n						
Person-time						
Incidence, per 1000 py						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Animal Protein Intake						
Events, n						
Person-time						
Incidence, per 1000 py						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Vegetable Protein Intake						
Events, n						
Person-time						
Incidence, per 1000 py						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					

*adjusted for age, sex, race, study, center, and total energy intake

** adjusted for age, sex, race, study center, total energy intake, smoking, education, systolic blood pressure, use of antihypertensive medication, HDLc, total cholesterol, use of lipid lowering medication, body mass index, waist-to-hip ratio, alcohol intake, sports-related physical activity, leisure-related physical activity, carbohydrate intake, fiber intake, and magnesium intake

*Table 3. Association of total, animal and vegetal protein intake with stroke subtypes (HR and 95%CI)**, ARIC 1987-2011*

	Q 1	Q 2	Q 3	Q 4	Q 5	p-trend
Total Protein Intake						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Animal Protein Intake						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Vegetable Protein Intake						
Hemorrhagic	1 (ref)					
Ischemic	1 (ref)					
Silent Stroke	1 (ref)					

** adjusted for age, sex, race, study center, total energy intake, smoking, education, systolic blood pressure, use of antihypertensive medication, HDLc, total cholesterol, use of lipid lowering medication, body mass index, waist-to-hip ratio, alcohol intake, sports-related physical activity, leisure-related physical activity, carbohydrate intake, fiber intake, and magnesium intake

Table 4. Association of major dietary protein sources with total (ischemic and hemorrhagic) stroke, ARIC 1987 – 2011

	Q 1	Q 2	Q 3	Q 4	Q 5	p-trend
Processed Meat						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Red Meat						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Red Meat & Processed Meat						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Poultry						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Dairy						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
High-Fat Dairy						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Low-Fat Dairy						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Fish & seafood						

Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Eggs						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Nuts						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					
Legumes						
Median svg/day						
HR (95%CI)*	1 (ref)					
HR (95%CI)**	1 (ref)					

*adjusted for age, sex, race, study, center, and total energy intake

** adjusted for age, sex, race, study center, total energy intake, smoking, education, systolic blood pressure, use of antihypertensive medication, HDLc, total cholesterol, use of lipid lowering medication, body mass index, waist-to-hip ratio, alcohol intake, sports-related physical activity, leisure-related physical activity, carbohydrate intake, fiber intake, and magnesium intake

Table 5. Association of major dietary protein sources with stroke subtypes (HR and 95% CI), ARIC 1987 – 2011**

	Q 1	Q 2	Q 3	Q 4	Q 5	p-trend
Processed Meat						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Red Meat						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Red Meat & Processed Meat						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Poultry						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Dairy						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
High-Fat Dairy						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Low-Fat Dairy						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Fish & seafood						

Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Eggs						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Nuts						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					
Legumes						
Hemorrhagic Stroke	1 (ref)					
Ischemic Stroke	1 (ref)					
Silent Stroke	1 (ref)					

**adjusted for age, sex, race, study center, total energy intake, smoking, education, systolic blood pressure, use of antihypertensive medication, HDLc, total cholesterol, use of lipid lowering medication, body mass index, waist-to-hip ratio, alcohol intake, sports-related physical activity, leisure-related physical activity, carbohydrate intake, fiber intake, and magnesium intake