Re: ARIC Manuscript Proposal:
"Dietary Protein Intake and Coronary Heart Disease in Middle-aged adults. Results from the ARIC Study"

Boston, September 2nd, 2010

Dear ARIC Publications Committee,

Hereby I am sending you my ARIC manuscript proposal for investigating "Dietary Protein Intake and Coronary Heart Disease in Middle-aged adults" using data from the Atherosclerosis Risk in Communities (ARIC).

The writing group consists of the following members: Bernhard Haring; Alvaro Alonso; Moritz Wyler von Ballmoos; Noelle Gronroos; Jennifer Nettleton; Elizabeth Selvin.

I attended the July2010 NHLBI workshop at Northwestern University.

Thanks a lot for your consideration and help!

Yours sincerely,

Bernhard Haring

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ARIC Manuscript Proposal # 1691

PC Reviewed: 9/14/10 **Status: A** **Priority: 2**
SC Reviewed: _____ **Status: _____** **Priority: ____**

1.a. Full Title:
"Dietary Protein Intake and Coronary Heart Disease in Middle-aged adults. Results from the ARIC Study"

b. Abbreviated Title (Length 26 characters):
Protein & Cardiovascular Risk

2. Writing Group:
Writing group members:
Bernhard Haring; Alvaro Alonso; Moritz Wyler von Ballmoos; Noelle Gronroos; Jennifer Nettleton; Elizabeth Selvin; 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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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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3. Timeline:
Data preparation and analysis will begin upon approval, and manuscript drafting will commence once suitable analytical models are finalized. Initial drafts will be circulated among the writing group members. The expected timeline is 2010 – 2011.

Rationale:
Over the past twenty years, a range of investigations have examined the effect of various diets differing in their macronutrient content (fat, carbohydrate, protein) on

cardiovascular health. In case of a protein rich diet, the source of protein intake was found to be influential on cardiovascular risk factors: Previous observational studies report an inverse association of total protein intake with cardiovascular risk factors, in particular systolic and diastolic blood pressure [1-6], but these results are conflicting [5, 7, 8]. Some evidence also exists for an inverse association between plant protein intake and blood pressure [6-9], however, these findings have not been consistent across studies [10]. On the other hand, a higher intake of red meat was reported to be associated with an increased risk of coronary heart disease [11]. In sum, these differing observational results regarding the significance of dietary protein sources for cardiovascular risk factors may reflect populational differences in dietary patterns (i.e. differing chief food sources of protein among study populations) and residual confounding deriving from unmeasured lifestyle specific factors. Thus, general conclusions regarding the effect of various sources of protein intake on cardiovascular health are difficult to draw. Furthermore, long-term follow-up studies including cardiovascular endpoints such as coronary heart disease are sparse [11] and a clear consistent understanding of the relation between various dietary protein sources (i.e. food groups) and the development of coronary heart disease has not been developed yet.

Hereby we propose to characterize and compare the relationships between various levels of protein (i.e. total, animal and vegetable protein intake) as well as different food sources of protein (i.e. total meat, red meat, poultry, fish, dairy, eggs, beans, nuts) and the risk for coronary heart disease in a large community-based cohort of middle-aged adults with long term follow-up who did not have a history of diabetes, hypertension, and other cardiovascular disease at baseline.

Study Question:

We aim to assess the association of different sources (dairy, total meat, red meat, poultry, fish, eggs, beans, nuts) and levels of protein intake (animal, vegetable and total protein intake) at baseline and follow-up with risk of coronary heart disease during 20 years of follow-up.

We hypothesize that high vegetable protein intake, as compared to animal and total protein intake will be associated with a lower risk for coronary heart disease in a community-based prospective cohort study. We further hypothesize that high red meat intake is associated with a higher risk for coronary heart disease in a community-based prospective cohort study.

Methods:

Study Population

We will analyze data from the Atherosclerosis Risk in Communities Study (ARIC). The ARIC study is a community-based prospective cohort study of 15,792 middle-aged adults (aged 45-64) from four U.S. communities. All participants received an extensive examination, including collection of medical, social, and demographic data. The first

examination of participants (visit 1 / baseline) took place during 1987–1989, with three follow-up visits taking place each approximately every 3 years with a fourth and last exam in 1996-98.

Inclusion / Exclusion Criteria
For our analysis we will include individuals of African-American or Caucasian background and exclude individuals with self-reported diabetes or use of diabetes medication, with self-reported hypertension or use of hypertension medication, or cardiovascular disease or a validated cardiovascular event at visit 1 or had missing data on covariates of interest (all data recorded during visit 1). Participants with incomplete dietary information or with extreme calories will be excluded from further analysis.

Assessment of protein intake (Exposure)
Dietary data on protein sources (dairy, total meat, red meat, poultry, fish, beans, eggs, nuts) were collected at visit 1 and 3 via a validated 66 item Food Frequency Questionnaire (FFQ) developed by Walter Willett. The Harvard Nutrition Database was used to assign nutrient values (specifically total protein and animal protein intake) to each FFQ line item. For analysis purposes, vegetable protein intake will be calculated as the difference of total and animal protein intake.

For assessing dietary behaviour, participants will use the average intake of various protein sources derived from the mean of all FFQ intakes received from information of all available FFQs (i.e visit 1 & 3 diet data for participants with follow-up after visit 3; prior to visit 3, only baseline data will be used). This approach is likely to reduce within-person variation and may best represent long-term dietary behavior.

Assessment of coronary heart disease (Outcome)
The primary end point for this study is coronary heart disease occurring after the return of the FFQ at visit 1. Coronary heart disease will be defined as a definite or probable myocardial infarction, a death from coronary heart disease, a cardiac procedure, or electrocardiographic evidence of a silent myocardial infarction [15, 16]. Information on coronary heart disease will enter the analysis starting with visit 1 (1987–1989) through the end of 2007 (or most recent data available).

Analysis:
We will evaluate the individual associations between the variables ‘total protein intake’, ‘animal protein intake’, as well as ‘vegetable protein intake’ and the risk of coronary heart disease. We will thereafter use information on the consumption of ‘total meat’ (i.e. computed grouped variable of chicken or turkey with and without skin, hamburger, hot dog, processed meats, bacon, beef/pork/lamb as a sandwich or main dish), ‘red meat’ (i.e. computed grouped variable of hamburger, beef, hot dog, processed meats, bacon, beef/pork/lamb as sandwich or main dish), ‘fish’ (i.e. computed grouped variable of tuna, other fish, and shrimp/lobster/etc.), ‘poultry’ (i.e. computed grouped variable of chicken or turkey with and without skin), ‘dairy’ (i.e. computed grouped variable of skim or low fat milk, whole milk, yogurt, ice cream, cottage cheese or ricotta cheese, other cheeses,

margarine, butter), ‘beans’ (i.e. computed grouped variable of string or green beans, baked beans or lentils), ‘eggs’ and ‘nuts’ in relations to the risk of coronary heart disease.[11]

First, we aim to characterize dietary behaviour of study participants according to quartiles of the cumulative average total protein intake. The average will be derived from the mean of all FFQ intakes received from information of all available FFQs (i.e. visit 1 & 3 diet data for participants with follow-up after visit 3; prior to visit 3, only baseline data will be used). [Table 1]

Person-years of follow up will be calculated from baseline examination at visit 1 to the date of the first coronary event.

Hazard ratios and corresponding 95% confidence intervals will be estimated with the use of Cox proportional-hazards regression modelling according to quartiles of total protein intake, vegetable protein intake and animal protein intake. Therefore, we will divide each quartile by the lowest quartile and adjust for age, sex and race (basic adjustment). Thereafter we will apply multivariable proportional hazard modelling and control for LDL, HDL, triglyceride level, BMI, waist-to-hip ratio, physical activity, smoking status, alcohol intake, total energy intake and total carbohydrates [11, 15, 16]. [Table 2]

Similarly, hazard ratios and corresponding 95% confidence intervals will be estimated with the use of Cox proportional-hazards regression modelling according to quartiles of different protein sources intake (dairy, total meat, red meat, poultry, fish, eggs, beans, nuts). Therefore we will divide each quartile by the lowest quartile and adjust for age, sex and race (basic adjustment). Thereafter we will apply multivariable proportional hazard modelling and control for LDL, HDL, triglyceride level, BMI, waist-to-hip ratio, physical activity, smoking status, alcohol intake, total energy intake and total carbohydrates [11, 15, 16]. [Table 3]

We will not include the diagnoses of diabetes mellitus and hypertension nor the associated use of treatment medications in our Cox proportional-hazards regression modelling since they are likely to be intermediate outcomes than true confounding factors.

Finally, we will investigate the potential effect of substituting one protein source for another (e.g. vegetable protein for animal protein, fish for red meat, etc.). [Table 4]

Table 1: Selected characteristics of the study participants according to quartiles of cumulative average total protein intake

	Low Protein Consumption 1st quartile	2nd quartile	3rd quartile	High Protein Consumption 4th quartile
Number of participants				
Median Total Protein Intake (g)				
Median Animal Protein(g)				
Median Vegetable Protein (g)				
Age (yr)				
Sex (%)				
Female				
Male				
Race (%)				
Black				
White				
Total cholesterol (mg/dl)				
LDL (mg/dl)				
HDL (mg/dl)				
Triglycerides (mg/dl)				
Glycated hemoglobin (%)				
Body-mass index				
Waist-to-hip ratio				
Blood Pressue (mmHg)				
Education (%)				
Less than high school				
High school or equivalent				
College or above				
Alcohol use (%)				
Currently				
Formerly				
Never				
Baecke's physical-activity index score				
Smoking status (%)				
Current smoker				
Former smoker				
Never smoked				

Table 2: RRs and 95% CIs for coronary heart disease according to quartiles of total protein intake, vegetable protein intake and animal protein intake

		Quartiles				P for trend	RR
		1st	2nd	3rd	4th		
Total Protein							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Animal Protein							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Vegetable Protein							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						

Basic adjustment for age, sex and race.
Multivariable model includes LDL, HDL, triglyceride level, BMI, waist-to-hip ratio, physical activity, smoking status, alcohol intake, total energy intake and total carbohydrates.

Table 3: RRs and 95% CIs for coronary heart disease according to quartiles of intake of different protein sources (dairy, total meat, red meat, poultry, fish, eggs, beans, nuts).

		Quartiles				P for trend	RR
		1st	2nd	3rd	4th		
Total Meat							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						

Red Meat							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Fish							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Poultry							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Eggs							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Dairy							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Beans							
	Median Intake per day						
	Basic Adjustment						
	Multivariable Adjustment						
Nuts							
	Median Intake per day						
	Basic Adjustment						
	Multivariable						

	Adjustment						

Basic adjustment for age, sex and race.
Multivariable model includes LDL, HDL, triglyceride level, BMI, waist-to-hip ratio, physical activity, smoking status, alcohol intake, total energy intake and total carbohydrates.

Table 4: RRs and 95% CIs for coronary heart disease of substituting one protein intake or source for another

	RR	95% CI
Vegetable protein for animal protein		
Poultry for red meat		
Nuts for red meat		
Fish for red meat		
Beans for red meat		
Dairy for red meat		
Dairy for fish		
Dairy for poultry		
Nuts for fish		
Beans for fish		
Nuts for poultry		
Beans for poultry		
Fish for poultry		

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

8.a. Will the DNA data be used in this manuscript? No
8.b. NA

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.
There is no overlap between this proposal and current proposals/published manuscripts.

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)?
1. Dietary Risk factors for decreased renal function in the ARIC study.
Lead Author: J Coresh / MS #224
2. Diet and Peripheral Arterial Disease: The ARIC Study.
Lead Author: ZJ Zheng / MS #302
3. Foods, dietary patterns, and prevalence of microalbuminuria in the Atherosclerosis, Plaque and CVD in Communities Study
Lead Author: JA Nettleton / MS# 1209
4. Dietary risk factors for 9-year incidence of decreased renal function in the ARIC Study.

Lead Author: J Coresh / MS #348
5. Relationship Between Dietary Protein Intake and Blood Pressure in Middle age Adults.
Lead Author: CY Wang / MS #461
6. Dietary fat and risk of CHD and stroke incidence and mortality in the ARIC study.
Lead Author: E Guallar / MS # 802

11.a. Is this manuscript proposal associated with any ARIC ancillary studies or does it use any ancillary study data? No
11.b. NA

12. 1-3 year completion expectation: 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.

Literature:

[1] Stamler J, Caggiula A, Grandits GA, Kjelsberg M, Cutler JA. Relationship to blood pressure of combinations of dietary macronutrients. Findings of the Multiple Risk Factor Intervention Trial (MRFIT). Circulation 1996;94:2417-2423.
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[4] Stamler J, Elliott P, Kesteloot H, Nichols R, Claeys G, Dyer AR, *et al.* Inverse relation of dietary protein markers with blood pressure. Findings for 10,020 men and women in the INTERSALT Study. INTERSALT Cooperative Research Group. INTERnational study of SALT and blood pressure. Circulation 1996;94:1629-1634.
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[10] Obarzanek E, Velletri PA, Cutler JA. Dietary protein and blood pressure. JAMA 1996;275:1598-1603.
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[12] Appel LJ, Sacks FM, Carey VJ, Obarzanek E, Swain JF, Miller ER, 3rd, *et al.* Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. JAMA 2005;294:2455-2464.

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[13] Carey VJ, Bishop L, Charleston J, Conlin P, Erlinger T, Laranjo N, *et al.* Rationale and design of the Optimal Macro-Nutrient Intake Heart Trial to Prevent Heart Disease (OMNI-Heart). Clin Trials 2005;2:529-537.
[14] Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. Am J Clin Nutr 1997;65:1220S-1228S; discussion 1229S-1231S.
[15] Selvin E, Steffes MW, Zhu H, Matsushita K, Wagenknecht L, Pankow J, *et al.* Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. N Engl J Med;362:800-811.
[16] Alonso A, Nettleton JA, Ix JH, de Boer IH, Folsom AR, Bidulescu A, *et al.* Dietary phosphorus, blood pressure, and incidence of hypertension in the atherosclerosis risk in communities study and the multi-ethnic study of atherosclerosis. Hypertension;55:776-784.