ARIC Manuscript Proposal # 1695

PC Reviewed: 9/14/10                             Status: A                             Priority: 2
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1.a. Full Title: Intake Of Fish-Derived N-3 Polyunsaturated Fatty Acids, Incidence of Diabetes, And Markers of Glucose Homeostasis In The Atherosclerosis Risk In Communities (ARIC) Study

b. Abbreviated Title (Length 26 characters): N-3 PUFAs and Glycemia

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I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. _nng_ [please confirm with your initials electronically or in writing]

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3. Timeline:
Statistical Analysis: September 2010 – December 2010
Manuscript Preparation: January 2011 – March 2011
Manuscript Revision: April 2011
Public health officials recommend consumption of non-fried, oily fish to promote cardiovascular health.\textsuperscript{1, 2} Intake of the fish-derived n-3 fatty acids DHA and EPA may also affect risk of T2D and markers of glucose homeostasis such as fasting insulin levels and HbA1c. The following are potential mechanisms through which fish-derived n-3 PUFAs may decrease insulin resistance:

1. A pro-inflammatory state interferes with insulin signaling and inhibits insulin action on adipocytes.\textsuperscript{3} Fish-derived n-3 PUFAs have anti-inflammatory properties and inhibit the production of pro-inflammatory cytokines\textsuperscript{4} and increase insulin sensitivity.\textsuperscript{5}

2. Fish-derived n-3 PUFAs are incorporated into cell membranes where they may modify the activities of membrane-associated enzymes and receptors\textsuperscript{6} and increase cell-sensitivity to insulin.\textsuperscript{4}

However, contradictory epidemiologic and clinical data exist on the association of n-3 PUFAs and the risk of diabetes and markers of glucose homeostasis. A Cochrane review considering fish oil supplement trials in diabetics showed that, overall, the n-3 fatty acids did not effect fasting blood glucose (FBG) levels, HbA1c, or fasting insulin.\textsuperscript{7} Fish oil trials in nondiabetic patients have been similarly null.\textsuperscript{8, 9}

Observational studies have provided inconsistent results. Fish and EPA/DHA intake have been positively associated with risk of T2D;\textsuperscript{10-13} associated with a reduction in risk of T2D\textsuperscript{14, 15} and lower FBG;\textsuperscript{16} or have had null associations.\textsuperscript{13, 17-19} These data may conflict for several reasons: (1) study duration: trials were short term compared to observational trials and there man not been enough time for the effect of fish oil supplements to be fully realized; (2) dose: trials used DHA and EPA doses that far exceed typical dietary intake; (3) study populations: different study populations with different fish preparation methods and cultural differences may not be comparable; and (4) different exposure levels: some studies had high levels of DHA and EPA intake (i.e., fish oil supplements) while others only investigated typical dietary consumption.

Our proposed study will determine if intake of fish and fish-derived n-3 fatty acids are associated with incident T2D, fasting blood glucose, HbA1c, oral glucose tolerance test (OGTT) post-challenge glucose measures, and fasting insulin separately in nondiabetics and diabetics in the ARIC study. Our proposed study is uniquely able to investigate the association of fish and fish-derived n-3 PUFA with glucose homeostasis as the ARIC dataset contains three different measures of fish-derived n-3 PUFA intake (exposures), several different measures of glucose homeostasis (outcomes), repeated measures over 9 years, and data on both diabetic and non-diabetic subjects.

5. Main Hypothesis/Study Questions:

We plan to assess the following aims in the ARIC study:

- **Specific Aim 1**: To estimate the prospective association of intake of fish and fish-derived n-3 PUFAs with incident T2D in nondiabetics.

- **Specific Aim 2**: Among nondiabetics, to estimate the cross-sectional associations of fish and fish-derived n-3 PUFAs with fasting insulin, fasting blood glucose (FBG), HbA1c, and post-OGTT glucose.
• **Specific Aim 3**: Among participants with prevalent diabetes, to estimate the cross-sectional associations of fish and fish-derived n-3 PUFAs with fasting insulin, FBG, and HbA1c.

Given that n-3 fatty acids are associated with biochemical changes that reduce insulin resistance, we hypothesize that a higher intake of n-3 PUFAs will be associated with a lower risk of developing diabetes, and lower levels of glucose, insulin, and HbA1C in both diabetics and nondiabetics.

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**: We propose to conduct a longitudinal prospective analysis of the ARIC study excluding all participants with a history of cardiovascular disease (CHD, heart failure, stroke) at baseline. Additionally, data from the Multi-Ethnic Study of Atherosclerosis may be included in later analyses to increase power. Figure 1 illustrates the exposures, outcomes, and study populations from each ARIC visit.

![Figure 1 – Exposures, outcomes, and study population by ARIC visit.](image)

**Study Population**: For specific aim 1, we will include nondiabetics at baseline, free of CVD. For specific aims 2 and 3, our unit of analysis will be participant-visits and not individual participants. At each study visit the study population will be updated to reflect those newly diagnosed with T2D and exclude those with CVD.

**Exposures**: Exposure to fish-derived n-3 PUFAs were assessed three ways in ARIC:

1. Oily fish, other (non-oily) fish, shellfish, and canned tuna intake (in servings/week) was measured with a food frequency questionnaire (FFQ) at visits 1 and 3
   a. We will consider three types of fish intake: total fish intake including shellfish, total fish intake without shellfish, and oily fish.
   b. Intake will be categorized into servings/week (0, <1, 1 to <2, and 2+).
2. Daily intake of DHA and EPA (in g/day) was derived from the FFQ
   a. We will consider DHA only, EPA only, and DHA+EPA
   b. Fatty acids will be categorized into quintiles
3. In the Minnesota field center only, plasma fatty acid concentration was measured in stored samples from the baseline visit as percent of total fatty acids
   a. We will consider DHA only, EPA only, and DHA+EPA
   b. Fatty acids will be categorized into quintiles

**Outcomes**: We will include the following 5 variables as outcomes:

1. Fasting blood glucose (measured at all four visits)
(2) Fasting insulin (measured at visits 1 and 4)
(3) HbA1c (measured at visit 2 only)
(4) Post-OGTT glycemia (measured at visit 4 only)
(5) Incident T2D (measured at all visits and during annual follow-ups after visit 4)
   a. At visits 1-4 T2D was defined as FBG>125 mg/dL, non-fasting blood glucose >200 mg/dL, self-reported physician diagnosis of T2D, or use of antidiabetic medication in any of the follow-up visits among individuals free of diabetes at baseline.
   b. After visit 4 T2D was defined as self-reported physician diagnosis of T2D.

• **Aim 1**: The outcome will be incident T2D

• **Aim 2**: The outcomes will be FBG, insulin, HbA1c, and post-OGTT

• **Aim 3**: The outcomes will be FBG, insulin, and HbA1c.
  o Post-challenge OGTT was not measured in a representative sample of diabetics

**Confounders**: In our analysis we will consider the following variables as potential confounders: BMI, weight gain, physical activity, alcohol intake, intake of cereal fiber, saturated fats and trans fats, total calorie intake, smoking, age, race, educational level, gender, and study center. Because BMI might be a mediator in the association of n-3 PUFAs with diabetes and markers of glucose homeostasis, we will conduct analysis with and without adjusting for this variable. Confounder values will be measured during the same visit as exposure values.

**Analyses**: The following details the analysis plan for each of the three aims.

**Aim 1 (nondiabetics):**
- We will use Cox proportional hazards regression with visit 1 dietary values (fish servings, DHA and EPA) as exposures and incident diabetes in at visits 2-4, and during annual follow-ups as the outcome.
- We will conduct an additional analysis updating dietary intake and confounders using data from visit 3 using Cox proportional hazards regression with time varying variables.

**Aim 2 (nondiabetics):**
- We will use mixed models to explore intake of fish/n-3 PUFAs in visits 1 and 3 with FBG measured at visits 1-4.
- We will use multiple linear regression to explore whether fish/n-3 PUFA intake measured at visit 1 is associated with insulin measured at visit 1 and HbA1c measured in visit 2, and whether fish/n-3 PUFA intake measured at visit 3 is associated with post-OGTT glycemia and insulin in visit 4.

**Aim 3 (diabetics):**
- We will use mixed models to explore intake of fish/n-3 PUFAs in visits 1 and 3 with FBG measured at visits 1-4.
- We will use multiple linear regression to explore whether fish/n-3 PUFA intake measured at visit 1 is associated with insulin measured at visit 1 and HbA1c measured in visit 2, and
whether fish/n-3 PUFA intake measured at visit 3 is associated with post-OGTT glycemia and insulin in visit 4.

• Additionally, subjects who take antidiabetic medications will have their outcome measurements adjusted to be comparable with diabetic subjects who are not on pharmacological therapy.20

Methodologic Issues: There are at least four limitations related to our study design:

1. Measurement error: fish/n3 PUFA intake will be assessed using a FFQ. Even though this questionnaire has been validated, measurement error is certain to exist.
2. As preparation technique is not available we will be unable to differential between fried fish and other types of fish.
3. Fasting was self-reported, so subjects may not have fasted for 8+ hours before FBG was measured. However, we can compare the FBG results to those obtained using HbA1c as HbA1c does not require fasting.
4. Data are limited on fish oil supplements. Although fish oil supplements were not widely used in the late 1980’s early 1990’s, at baseline there were n=108 fish oil supplement users in the MN cohort (2.7%). Additionally, a vitamin survey (including fish oil supplements) was conducted during visit 3. Sensitivity analyses will be performed to determine if inclusion or exclusion of these subjects affect the measures of association.
5. In many cases exposures were measured three years before outcomes (e.g., diet at visit 1 and HbA1c at visit 2, diet at visit 3 and post-OGTT at visit 4). This delay between exposure measurement and outcome may create bias.

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 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 ICTDER03 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 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.csec.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)?

#197 Medication, diet & diabetes

#282 Dietary Fat – Insulin

#931 Dietary fat consumption and incidence of type 2 diabetes

#989 Associations of plant- and animal-based food consumption with risk of developing type 2 diabetes: the Atherosclerosis Risk in Communities (ARIC) Study

#1173r Dietary intake and the development of the metabolic syndrome: The ARIC study.

#737 Dietary intake as a predictor of incidence of type 2 diabetes in African-Americans (AAs) and Whites.

#1618 Does physical activity modify the association between plasma fatty acids and incident Type 2 diabetes: The Atherosclerosis Risk in Communities (ARIC) study?

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

11.b. If yes, is the proposal

__X__  A. primarily the result of an ancillary study (list number* _2006.15__)

___  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.cscnc.unc.edu/aric/forms/

12. 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.

References:

