1.a. Full Title: Associations between diet patterns and diabetic retinopathy in a biracial cohort

b. Abbreviated Title (Length 26 characters): Diet and diabetic retinopathy

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

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3. Timeline:

Analyses are planned to be completed between December 2014 and May 2015.
4. Rationale:

**Diabetic retinopathy**

As dramatic global increases in the prevalence of diabetes are taking place, complications due to diabetes can be expected to increase as well.(1, 2) Currently an estimated 93 million people have some type of diabetic retinopathy globally (~35% of all people with diabetes) and ~10% had retinopathy advanced enough to endanger their vision.(3, 4) Poor blood glucose control, high blood pressure and longer duration of diabetes have been shown to be risk factors for diabetic retinopathy(5), however, identification of additional modifiable risk factors such as diet may help reduce the burden of diabetic retinopathy. Diet has been relatively understudied with respect to diabetic retinopathy in epidemiologic studies.(6)

**Diabetic retinopathy, oxidative stress and inflammation**

Individuals with diabetes are thought to be in an increased state of systemic oxidative stress (7) and inflammation (8). The retina, with its high proportion of polyunsaturated fatty acids and light exposure (9), is highly susceptible to oxidative stress. This insult is likely further exacerbated in individuals with diabetes due to the presence of such factors as hyperglycemia, hypertension and hyperlipidemia. Hyperglycemia can lead to retinal microvascular damage through increased permeability due to disruption of the blood retinal barrier, loss of pericytes, increased production of endothelial cells and neovascularization.(10) Hyperglycemia elicits inflammation and the seepage of fluid from the blood vessels into the surrounding tissue and recruitment of leukocytes to the area of damaged tissue.(11) These processes may result in blocked capillaries and decreased retinal blood flow. (12) hypoxia and the resultant angiogenesis seen in diabetic retinopathy. (10, 13) Along with pathways involving hypertension and hyperlipidemia, hyperglycemia elicits oxidative stress, inflammation, cell death or a combination of these factors that may induce damage to cells in the retinal vasculature, the cumulative effect of which may be severe enough to cause loss of sight.(7, 10)

**Dietary patterns may influence risk of diabetic retinopathy**

Diet is a well-established risk factor for inflammation.(14-16). In epidemiological studies, markers of inflammation have been shown to be positively associated with saturated and trans fats and inversely associated with such nutrients as omega-3 fatty acids, fiber, antioxidants, vitamins A and D, certain minerals (selenium and iron), as well as moderate intake of ethanol.(16) Studies have shown that adherence to diets rich in fruits, vegetables, legumes, whole grains, poultry and fish and low in fried and processed foods, as well as refined grains and sugars, is inversely associated with presence of inflammatory markers.(16)

Diet has also been shown to be associated with oxidative stress. Studies have found a beneficial effect of consuming fruit and vegetables, and monounsaturated fatty acid rich foods on oxidative stress, while intake of saturated fatty acids and alcohol seemed to have an unfavorable effect. (17) Observational studies examining certain dietary patterns such as the Mediterranean, the DASH (Dietary Approaches to Stop Hypertension) and a high total antioxidant capacity diet suggest that the synergistic effects of foods eaten together is likely beneficial.(17)

Despite a compelling rationale for the role of dietary intake in development or progression of diabetic retinopathy, limited epidemiologic data exist on associations between diet and diabetic retinopathy. Some studies have examined associations between single nutrients and diabetic retinopathy, but not enough work has been done to be able to make conclusions regarding dietary exposures and disease risk.(6) Further, very little work has been conducted on overall dietary patterns and diabetic retinopathy. It may be beneficial to investigate patterns of dietary intake in relation to disease in addition to that of single nutrients. The magnitude of an association between a nutrient and a disease may be too small to measure, but when the effects of multiple nutrients are added together, as in a dietary pattern, the association may be larger and easier to observe.(18, 19) There can be synergistic or antagonistic effects between nutrients which are sometimes found together. These effects may not be evident if a nutrient is studied without considering it alongside other nutrients. (18-21) Moreover, when a single nutrient or food is added to the diet, caloric intake may increase unless additional changes are made to the
diet already being consumed (22) or the adjustment to the individual’s habitual diet may result in removal of other important nutrients or foods from their diet. Investigating food consumption using dietary patterns enables us to look at nutrition-disease associations using food consumption as it occurs in the real world.(18)

Few studies have examined associations between overall diet and the development and progression of retinopathy. Adherence to a Mediterranean diet pattern has been associated with greater total antioxidant capacity(23) and better blood glucose control, a known risk factor for retinopathy.(24) Individuals who participate in interventions to increase fiber intake or introduced a vegetarian diet were more likely to have reduced HbA1c levels as well as blood lipid levels and body weight (other factors thought to be associated with diabetic retinopathy) than controls.(25) A case control study conducted in India found that controls, without proliferative diabetic retinopathy, were more likely to adhere to a traditional diet high in pulses, fruits and vegetables than cases, who were more likely to follow a diet higher in animal products and fats. (26) In China, a twenty-year follow-up of a randomized controlled trial in people with impaired glucose intolerance found a decreased incidence of severe retinopathy in those who were assigned to a six year intervention aimed to decrease alcohol and sugar use and increase vegetable intake, compared to controls (hazard ratio = 0.53, 95% CI: 0.29-0.99).(27) A study conducted among individuals with diabetes in Australia found that migrants from Greece, who may be more likely to adhere to a Mediterranean diet, were at lower risk of retinopathy than Australians.(28) A cross sectional analysis in the Democratic Republic of Congo revealed that people with diabetes who consumed a diet similar to the Mediterranean diet were significantly less likely to have cataract, glaucoma or blindness than those who did not. Although it did not examine “diabetic retinopathy” per se. (29) Further research to better understand the association between diet quality and diabetic retinopathy is needed.

In the Atherosclerosis Risk in Communities (ARIC) Study we have the opportunity to investigate associations between diet (assessed from 1987-89), and diabetic retinopathy (assessed from 1993-96) in a large population-based cohort of Caucasian and African American men and women with primarily Type 2 diabetes (n=1,430). Diabetic retinopathy was assessed from grading of a single fundus photograph of one randomly chosen eye taken approximately three years after the collection of dietary intake. We also have the availability of numerous demographic, medical and lifestyle variables to explore as potential confounding factors or effect modifiers of this relationship.

5. Main Hypothesis/Study Questions:

Main Study Question
Is there an association between adherence to a healthy diet pattern, assessed at visit 1 (1987-1989), and the presence of diabetic retinopathy assessed at visit 3 (1993-1995) among 1,430 individuals with prevalent diabetes at visit 3?

Hypothesis:
The odds of retinopathy are higher in those with low, as compared to high, adherence to a healthy diet pattern score.

Exploratory Aim:
Is there an association between adherence to diet patterns as defined by reduced rank regression assessed at visit 1 (1987-1989), and the presence of diabetic retinopathy assessed at visit 3 (1993-1995) among 1,430 individuals with prevalent diabetes at visit 3?

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).
Sample
Our study sample will include those participants categorized by ARIC as having diabetes at visit 3 with gradable retinal photographs and having the ARIC 66-item food frequency questionnaire at visit 1 (n=1430). Participants were categorized as having diabetes if they had a non-fasting blood glucose concentration ≥200 mg/dl, a fasting blood glucose concentration of ≥ 126mg/dl, reported being told by a physician that they had diabetes and/or were on blood glucose lowering medication in the two weeks prior to the study visit.(30)

Disease endpoints
Prevalent retinopathy was determined from grading of retinal photographs taken at visit 3 (1993-1995) of one randomly selected eye. Participants sat in a dark room for 5 minutes to allow for nonpharmacological pupil dilation.(31) Retinal photographs were graded for the presence and severity of DR at the University of Wisconsin Fundus Photograph Reading Center using a standard grading system for participants, the modified Airlie House classification scheme (32). The eyes were graded for presence or absence of DR as well as its severity when present.

Assessment degree of adherence to a healthy diet pattern
At ARIC study visit 1, dietary data was collected by an interviewer administered questionnaire. This “Dietary Intake Form”, inquired about diet habits and included a 66 food frequency questionnaire (FFQ) which was adapted from a 61 food frequency questionnaire developed by Willet et al. to include additional questions about fish consumption and cooking fats.(33) This FFQ had been previously validated by Willett et al. in 173 women from the Nurse’s Health Study. (34)

Using this FFQ, an a priori Healthy Diet Score (HDS) will be calculated for each participant as outlined in Table 1 below. This score is based on the Alternate Mediterranean Diet Score creating by Fung et al. (35) which was found to be associated with a decrease in inflammation, a pathway through which diet may protect against diabetic retinopathy. This score also gives points for foods, such as fruits and vegetables, which have antioxidant properties and may protect against diabetic retinopathy. We have added one beneficial category (dairy) and four detrimental categories (sodium, sugar sweetened beverages and candy, fried foods, and refined grains and baked goods) to the score to reflect common dietary habits of persons residing in the United States. In order to use a larger range data, we will assign points based on the quintile into which consumption falls instead of assigning points based on the median amount consumed. Points assigned to each participant will be summed to create a score, higher scores reflecting a greater adherence to a healthy diet.

| Table1. Points assigned for each category of the Healthy Diet Score |
|-----------------------------------|-------------------|
| Category                          | Points assigned according to quintile of intake |
| **Beneficial**                    |                                  |
| Vegetables                        |                                  |
| Legumes                           | Quintile 1 = 0 points |
| Fruits                            | Quintile 2 = 1 points |
| Nuts                              | Quintile 3 = 2 points |
| Whole grain food                  | Quintile 4 = 3 points |
| Fish                              | Quintile 5 = 4 points |
| Fat ratio (PUFA/SFA)              |                                  |
| Dairy products                    |                                  |
| **Detrimental**                   |                                  |
| Sodium                            | Quintile 1 = 4 points |
| Sugar sweetened beverages and candy| Quintile 2 = 3 points |
| Fried foods                       | Quintile 3 = 2 points |
| Refined grains and baked goods    | Quintile 4 = 1 points |
| Meat and poultry                  | Quintile 5 = 0 points |
| Ethanol intake                    | Men - 4 points for consuming between 10 and 50 g/day |
|                                   | Women - 4 points for consuming between 2 and 25 g/day |

*Table 1: Points assigned for each category of the Healthy Diet Score*
Proposed analysis

Assessing the distribution of characteristics in the study sample
We will examine and test for differences in the distribution of characteristics and potential risk factors in the study sample according to HDS and prevalence of retinopathy. Categories of HDS will be made after examining the distribution of the HDS in the study sample. Analyses of the distribution of covariates within the study sample by HDS and prevalence of diabetic retinopathy will be performed using chi-square tests for categorical variables and t-tests or ANOVAs for continuous variables. Differences in the distribution of these variables will be considered significant at a p-value of <0.05.

Analysis of the association between HDS and prevalence of retinopathy
The association between HDS and prevalence of retinopathy will be investigated using logistic regression. We will first create a univariate model using HDS as the independent variable and retinopathy (no = 0 and yes = 1) as the dependent variable and calculate crude odds ratios (OR) and 95% confidence intervals (95% CI) comparing the highest category of HDS to the lowest category of HDS. We will consider those covariates that differ between groups by both HDS and prevalence of retinopathy at the ≤0.20 α level as potential confounders with the exception of blood pressure and HbA1c since these are at least partially in the causal pathway between HDS and retinopathy. These covariates will be evaluated separately as noted below. The adjusted model to be used in our analyses will be fit using a stepwise process where potential confounders that change the odds ratio by 10% or more are retained. We will present a crude model and a multivariate model adjusted for identified confounders. In order to investigate the degree to which the estimated association can be explained by blood pressure and/or glucose control, three additional models will be created:

- Multivariable adjusted model plus blood pressure
- Multivariable adjusted model plus HbA1c
- Multivariable adjusted model plus blood pressure, HbA1c and interaction term (blood pressure x HbA1c)

Exploratory analysis diet patterns using reduced rank regression
A priori diet scores, such as the HDS, have some limitations since they are subjective in their construction, assume that all categories contribute equally to the outcome (19) and ignore the way consumption of foods and nutrients are correlated. (36) For this reason, we will explore the association between diet patterns and retinopathy with reduced rank regression (RRR) (or maximum redundancy analysis) using data collected from the FFQ to create factors (i.e. groups of foods) that describe variation in response or intermediary variables and takes into consideration the correlation structure of the data.(19, 36-38) As has been done in other nutritional epidemiological studies, as responses in the RRR, we will consider variables that are associated with inflammation (white blood cell count) or known risk factors (blood glucose levels, blood pressure) for retinopathy which were assessed at visit 1. (19, 36, 39, 40) Because reduced rank regression extracts factors that describe variation in response variables, which are chosen due to previously established associations with retinopathy, we believe that it will be an improvement over principal components analysis which would only explain variation between people in predictor variables (i.e., food line items). We will calculate a score for each participant on each factor. Categories of the scores will be made after examining the distribution of these scores in the study sample.

We will conduct the analysis of the association between each factor and retinopathy in a manner similar to that of HDS except for the addition of blood pressure and HbA1c to the fully adjusted model. By examining dietary patterns defined using two different approaches, an a priori and a posteriori, we hope to better understand the association between diet and retinopathy in this sample.

Limitations and possible solutions
Although we will be using dietary data and data on potential confounders collected at the first ARIC study visit ~6 years prior to the assessment of diabetic retinopathy, temporality cannot be established since participants’ retinopathy status was unknown at baseline. Since participants who had begun to experience complications of diabetes may have changed their diet prior to the ARIC study’s assessment of retinopathy may have changed their
diet due to these complications we will conduct a sensitivity analysis which will exclude those participants (~653 or ~46%) who self-reported a diagnosis of diabetes prior to visit 1 or had taken medications for diabetes in the two weeks prior to study visit 1. We will examine the association between diet patterns and retinopathy using FFQ data collected at visit 3 as well as an average of dietary intakes assessed at visits 1 and 3. It has been suggested that an estimate of nutrient intake may have less measurement error when obtained by averaging data from multiple FFQs than one FFQ alone. (38, 45) Additionally, in order to investigate this association in participants with more stable dietary intakes, we will perform our analyses using data from only those participants who remained in the same or adjacent quartile of diet pattern score from visit 1 to visit 3.

Since this is a secondary data analysis our power to detect a significant association may be limited. Although we plan on examining the distribution of the dietary pattern scores before categorizing them, we conducted a power calculation which assumes that scores were categorized by quartiles and found that we would have approximately 79% power to detect an odds ratio of 0.85 for quartile one as compared to quartile four and 43% power to detect an odds ratio of 0.90 (α=0.05). Additionally, we feel that insight into the association between diet patterns and retinopathy may be gained even in the absence of statistically significant findings and may provide the impetus for future studies powered to detect such an association.

Another limitation of our data is the availability of retinal photographs in only one eye using film at visit 3 for classification of retinal eye disease. Therefore, there may be misclassification of retinopathy status. As the eye chosen to be photographed at visit 3 was done so randomly, we would expect non-differential misclassification of our endpoint which would bias our observed risk estimates toward the null.

7.a. Will the data be used for non-CVD analysis in this manuscript?  ____X__ Yes  ____ 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?

   ____X__ 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”?

   ____X__ 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.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)? Other relevant proposals are those that focus on diabetic retinopathy and would involve Dr. Ronald Klein. Dr. Klein is a co-author on this work.
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

   ___ A. primarily the result of an ancillary study (list number* 2010.20)
   __X__ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s)* 2006.15)

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12a. 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.

12b. 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.

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