ARIC Manuscript Proposal #2066

PC Reviewed: 2/12/13	**Status: <u>A</u>**	**Priority: <u>2</u>**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: Associations between vitamin D status and diabetic retinopathy in a biracial cohort

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

2. Writing Group:
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All writing group members are co-investigators, consultants, programmers or research assistants on the R01 (ARIC Ancillary Study 2010.20) funding this project "Vitamin D Status and Retinal Diseases in Aging."

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

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3. Timeline:

Analyses are planned to be completed between January 2013 and March 2014.

4. Rationale:

Diabetic retinopathy

In 2010 it was estimated that 12.3% of the US population aged 20-79 years have diabetes and this will increase to 14.0% by 2030 (1). A common complication of individuals with diabetes is diabetic retinopathy which is the leading cause of blindness in U.S. working aged adults (20-74 years) (2). Early retinopathy is defined by the presence of retinal microaneurysms, blot hemorrhage and hard exudate formation and termed non-proliferative diabetic retinopathy (NPDR). Later stages of disease exhibit retinal angiogenesis, bleeding and blindness (3) concomitantly with retinal neuronal and glial damage (4) and is termed proliferative diabetic retinopathy (PDR). Retinopathy affects ~40.3% of people with diabetes aged 40 years and older and is vision threatening in ~8.2% (1 in 12 persons) (5). Identifying modifiable risk factors and effective means of treatment to prevent or slow progression of retinopathy is of significant public health importance to preserving individual's quality of life and functional independence.

It has been hypothesized that a person's vitamin D status may affect whether or not he or she develops early-stage, or progresses to later-stages of diabetic retinopathy. Vitamin D is a fat soluble vitamin and is obtained from diet, supplements and sunlight. Humans can synthesize vitamin D in from a cholesterol precursor found in their skin upon exposure to ultraviolet B radiation. The major circulating and storage form of vitamin D is 25-hydroxyvitamin D (25(OH)D), which reflects intake from all sources. 25(OH)D is the preferred biomarker to assess vitamin D status because it reflects intake over the last 3 weeks unlike the active hormone, 1,25 di-hydroxyvitamin D (1,25(OH)$_2$D), which is highly regulated and has a half-life of ~4 hours (reviewed in (6)). The active hormone, 1,25(OH)$_2$D, binds to the vitamin D receptor (VDR), promoting heterodimerization of the VDR with the retinoid X receptor (RXR) (7). The VDR is expressed in vertebrate retina tissue (8, 9) and expressed in human cultured retinal endothelial cells (10). Vitamin D's proposed effects on ocular health are via the ligand/VDR/RXR complex's transcription factor activities (7) which affect the expression of genes involved in maintaining a multitude of biological processes (11, 12). Vitamin D is hypothesized to prevent against development or progression of retinopathy due to its anti-inflammatory, anti-angiogenic, and anti-hypertensive effects as well as its hypothesized benefit on blood glucose control and insulin sensitivity.

Studies of retinal microvascular endothelial cells (13) and animal models of diabetes (14) suggest that chronic low grade inflammation plays a role in the development of diabetic retinopathy (15). High blood glucose is thought to increased adhesion of leukocytes to microvascular endothelial cells leading to cell damage and impaired blood flow (14, 16) and consequential retinopathy lesions (4, 17). In humans, some (18-20) but not all (21-23) studies have shown associations between retinopathy and systemic markers of inflammation. Additionally, vitreous concentrations of cytokines have been found to be higher in patients with proliferative retinopathy compared to persons without retinopathy (24-26). Vitamin D may protect against retinopathy via its anti-inflammatory properties. VDR is expressed on cells of the human immune system (27-32). 1,25(OH)$_2$D, has been shown to suppress pro-inflammatory cytokines and toxic agents *in vitro* (33-35), and increase activities of T-helper 2 cells (anti-inflammatory) and suppress T-helper 1 cell (pro-inflammatory) response (36, 37), as well as to reduce the damaging effects of AGEs,

thought to induce an inflammatory response, in cultured endothelial cells (38). In this way, vitamin D may help prevent or ameliorate the inflammatory state that may promote retinopathy.

Proliferative diabetic retinopathy involves retinal angiogenesis, leading to retinal bleeding and loss of vision. Vitamin D has also been shown to inhibit angiogenesis in cultured endothelial cells (39), animal models of retinoblastoma (40) and oxygen-induced ischemic retinopathy (41), suggesting that being of a sufficient vitamin D status, as compared to being deficient for vitamin D, could prevent proliferative retinopathy.

Hypertension is an established risk factor for diabetic retinopathy (42). Recent evidence in human observational and clinical trials suggests that vitamin D is involved in blood pressure maintenance (43). Furthermore, studies in VDR knockout mice suggest that vitamin D regulates the Renin-Angiotensin System (44), and studies in 1 α-hydroxylase knockout mice (45) show that increased blood pressure is independent of calcium and phosphorus and stabilized with $1,25(OH)_2D$, suggesting vitamin D suppresses renin expression. Thus, deficiency of vitamin D may impact the renal system's influence on hypertension and related complications such as diabetic retinopathy.

Hyperglycemia is also an established risk factor for diabetic retinopathy (46). Epidemiologic studies have observed inverse associations between vitamin D status and markers of blood glucose control ((47, 48) and reviewed in (49)). The VDR is expressed in human pancreatic beta-cells (50) and $1,25(OH)_2D$ has been shown to increase insulin secretion in vitamin D deficient rats (51), suggesting that vitamin D deficiency may lead to poor blood glucose control. Sufficient vitamin D status could help prevent development of retinopathy if adequate vitamin D status helps maintain good blood glucose control.

Epidemiologic Evidence of Vitamin D Status and Diabetic Retinopathy

Compelling research in animal models (oxygen-induced ischemic retinopathy (41)) suggest that vitamin D may be protective against diabetic retinopathy, but epidemiologic data on the relationship between vitamin D status and retinopathy are limited. Two small clinical studies have investigated associations between vitamin D status and retinopathy. A study in 66 participants with noninsulin dependent diabetes (52) found lower concentrations of serum $1,25(OH)_2D$, but not 25(OH)D concentrations, among patients with pre-proliferative and proliferative diabetic retinopathy compared to patients with no or background retinopathy. A different paper presented data examining associations between diabetic retinopathy and serum 25(OH)D concentrations in 221 patients with and without diabetes (53). Payne et al. observed significant differences in vitamin D insufficiency (defined as <75 nmol/L) between study groups characterized by diabetes status and presence or absence of retinopathy, with the greatest percentage of deficient individuals (81%) among those with proliferative retinopathy. These associations in clinical studies could reflect changes in vitamin D status subsequent to the development of retinal disease and were not adjusted for numerous confounding influences.

In a previously conducted cross-sectional analysis in 581 Japanese men and women outpatients with type 2 diabetics, statistically significantly lower 25(OH)D concentrations were observed in participants with proliferative versus no diabetic retinopathy (48). In a multivariate analysis adjusted for potential confounding factors, researchers observed a statistically significant association between decreasing serum 25(OH)D and number of microvascular complications. No multivariate analyses were conducted for diabetic retinopathy alone. A recent paper using the Third National Health and Nutrition Examination Survey (NHANES III) showed statistically significantly increasing proportions of individuals with vitamin D deficiency with increasing

retinopathy severity, with the biggest differences observed in those with proliferative diabetic retinopathy (54). However, these analyses were not presented by race group or adjusted for season of blood draw. Differently, in a recently published, 26-year follow-up study of 220 patients with Type 1 diabetes attending a diabetes center, no associations were observed between plasma 25(OH)D concentrations and incidence of either background or proliferative diabetic retinopathy in adjusted analyses (47). However, this study was limited by its sample size and lack of data on season of blood draw which influences vitamin D status. All noted studies, except NHANES III, diagnosed retinopathy through eye examinations, not graded retinal photographs.

In the Atherosclerosis Risk in Communities (ARIC) Study we have the opportunity to investigate associations between serum 25(OH)D status and diabetic retinopathy in a population-based cohort of Caucasian and African American men and women with primarily Type 2 diabetes (n=1,612). Diabetic retinopathy was assessed from graded fundus photographs. Further, we have the availability of numerous medical and lifestyle variables to explore as potential confounding factors of the observed relationship.

5.   Main Hypothesis/Study Questions:

Main Study Question
Is there an association between vitamin D status, assessed at Visit 2 (1990-1992), and the presence of diabetic retinopathy assessed at Visit 3 (1993-1995) among 1,612 individuals with prevalent diabetes at Visit 3?

Main Hypothesis
We hypothesize that among ARIC participants with serum 25(OD)D measurements at visit 2 and prevalent diabetes and diabetic retinopathy assessment at visit 3, those with adequate (>50 nmol/L) compared to those with deficient or inadequate vitamin D status, based on concentrations of 25(OH)D at Visit 2, will have lower odds ratios for retinopathy.

Additional Study Questions
Are blood pressure or glycosylated hemoglobin pathway variables in the association between vitamin D and diabetic retinopathy?

Does race, gender, or blood glucose control modify the association between vitamin D and diabetic retinopathy?

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).

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 nonpharmocological pupil dilution (55). One 45-degree nonmydriatic retinal photograph was taken with a Canon CR-45UAF nonmydriatic film camera (Canon USA, Itasca, IL) and was centered to include the optic disc and the macula (55). 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 Arlie House classification scheme (56). The eyes were categorized into NPDR and PDR. Our main aim is powered to investigate the

outcomes of any diabetic retinopathy and NPDR. However because we have data on more detailed categorization of these diseases, associations of vitamin D and the presence of mild NPDR, moderate NPDR, and PDR will be explored. There are 345 individuals out of the 1,612 persons with diabetes who have retinopathy. Of these, 259 have mild NPDR, 53 have moderate to severe PDR, and 33 have PDR.

Assessment of blood vitamin D status

Serum was obtained at ARIC Visit 2 (1990-92) will be used to assess 25(OH)D with liquid chromatography/tandem mass spectrometry at the Collaborative Studies Clinical Laboratory at Fairview University Medical Center (Minneapolis, MN). All vitamin D assays are covered by Dr. Lutsey's R01 (Lutsey: R01 HL103706).

Proposed analysis

Participant characteristics and risk factors for diabetic retinopathy will be examined by vitamin D status as well as by severity level of diabetic retinopathy in order to identify potential confounders of the association between vitamin D status and diabetic retinopathy. Characteristics and risk factors measured at Visit 2, at the same time point as the exposure of serum 25(OH)D, will be used if possible. If this is not possible, risk factors available at Visit 1, as in the case of physical activity, will be used. If a risk factor is not available at Visits 1 or 2, then risk factor data from Visit 3 will be used. We will also explore whether composite variables using data from all multiple Visits, if available, (e.g., 6 year average in systolic blood pressure) might be a stronger risk factor for retinopathy than a risk factor measured at any one time point. Differences in characteristics by level of serum 25(OH)D and severity of retinopathy will be tested using t-t-tests, ANOVAs or chi-square tests depending on the nature of the variable being compared (e.g., continuous vs. categorical). A table will be presented showing characteristics by level of serum 25(OH)D concentrations.

Logistic regression models will be used to estimate the association between the log odds for any prevalent retinopathy in participants with adequate (≥50 nmol/L) compared to deficient or inadequate 25(OH)D concentrations (<50 nmol/L). Odds ratios (OR) and 95% confidence intervals (95% CIs) will be reported. In addition to testing the pre-specified threshold (50 nmol/L), exploratory analyses will be conducted to investigate the optimal thresholds of 30 nmol/L and 75 nmol/L and 25(OH)D will as be modeled as a continuous variable. Cubic splines will be utilized if necessary, to help us determine the most appropriate representation of the dose-response between 25(OH)D concentrations and diabetic retinopathy. Further exploratory analyses will investigate results for subgroups of NPDR as well as PDR. Lastly, we will explore associations between vitamin D status and severity of diabetic retinopathy using ordinal logistic regression.

Table 1 shows that we have adequate power (≥80%) to see effect sizes of 0.65 or greater.

Table 1: Power (%) to detect noted odds ratios when comparing the odds of NPDR among participants with adequate (25(OH)D >50 nmol/L) compared to deficient or inadequate (25(OH)D ≤50 nmol/L) vitamin D status.*							
Odds Ratio	0.60	0.65	0.70	0.75	0.80	0.85	0.90
Prevalent NPDR (Non proliferative diabetic retinopathy) (n=272) at Visit 3 (V3) in 1,600 individuals with diabetes	96%	87%	72%	53%	35%	20%	10%
*We assumed that 35% of the ARIC cohort would have 25(OH)D concentrations <50 nmol/L based on previous observations							

In multivariate modeling we will investigate potential confounding of our observed crude associations. These include age, race, duration of diabetes, blood cholesterol and triglycerides, hematocrit, smoking status, drinking status, physical activity, and body mass index (BMI). We will add potential confounders to our model in a step-wise fashion. Potential confounders will be added to the model if they change the OR 10% or more. The potential confounder that influences the crude OR to the greatest extent ≥10% will be adjusted for first. Next, all other potential confounders will be added singly to the expanded model, and the second most influential potential confounder will be added to the expanded model. This step-wise process will continue until no potential confounders influence the OR ≥10%. After development of the multivariate model for diabetic retinopathy we will explore the effect of addition of blood pressure as well as glycosylated hemoglobin as pathway variables to the multivariate model. If the relationship between vitamin D and diabetic retinopathy is attenuated this may suggest that vitamin D's effects on diabetic retinopathy may be mediated through vitamin D's influence on blood pressure or blood glucose control. Crude and adjusted models will be presented and adjusted models will be presented with and without pathway variables.

We will explore effect modification of the vitamin D and diabetic retinopathy associations by gender, race, and blood glucose control as measured by glycosylated hemoglobin. We will test for interaction by adding interaction terms to our logistic regression models. A p-value <0.10 for the interaction term will be considered statistically significant. If significant interactions are present, stratified results will be reported, though interpretation will be guided by the cell sizes and precision of effect estimates. We acknowledge that power to evaluate effect modification by gender, race is reduced compared to the power to investigate the main effects and therefore is exploratory.

Limitations and possible solutions
Our study is limited by its cross-sectional design. 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 endpoints ascertained at Visit 3. 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. We have digital retinal photos in both eyes at Visit 5 and will be able to assess the degree of misclassification of these retinal diseases in the ARIC cohort participants if only one of the two eyes was used for determination of disease. This information will be used to investigate the effect of misclassification on our risk estimates. It is also possible that the sensitivity of digital and film for the detection of diabetic retinopathy differ. However, we have previously shown film and digital grading of diabetic retinopathy to be comparable (57).

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)?
The most related manuscript proposals would be those involving Pam Lutsey's work on vitamin D and cardiovascular disease. Other relevant proposals are those that focus on diabetic retinopathy and would involve Dr. Ronald Klein. Both Drs. Lutsey and Klein are co-authors 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
  _X__ A. primarily the result of an ancillary study (list number* 2010.20)
  ___   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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