**ARIC Manuscript Proposal #2473**

1.a. **Full Title**: Association between Dietary Xanthophyll Intake and Prevalent Early Age-Related Macular Degeneration

b. **Abbreviated Title (Length 26 characters)**: Xanthophylls, HDL, and AMD

2. **Writing Group**:

Writing group members:

*University at Buffalo*: Henry Lin, BS (UB*) (paper lead), Amy Millen, PhD (UB), Michael LaMonte, PhD (UB), William Brady, PhD (UB), Michelle Sahli, MS (UB)

*University of Wisconsin*: Ronald Klein, MD (UW*), Barbara Klein, MD, (UW), Julie Mares, PhD, (UW), Kirstin Meyers, PhD (UW)

*Institution:  UB=University at Buffalo, UW=University of Wisconsin-Madison,*

All writing group members, with the exception of Dr. Brady (statistician) and Henry Lin, 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.” Henry Lin is a master’s student in the Department of Epidemiology and Environmental Health (advised by Dr. Millen) as well as an MD/PhD candidate at UB.

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

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3. **Timeline**: Analyses are planned to be completed between April and June 2015.
4. **Rationale:**

**Age-related macular degeneration**

Age-related macular degeneration (AMD) is the third leading cause of blindness worldwide, accounting for 5% of total blindness in 2010. A recent meta-analysis estimated the global prevalence of AMD to be 8.7%, and projected that the burden of disease will rise exponentially over the next few decades given the current trends of population aging. Moreover, in industrialized regions with better access to eye care and the lowest proportions of blindness, AMD remains the foremost cause of severe visual impairment in older adults, and approximately 50% of blindness may be attributable to AMD.

AMD is characterized by the formation of drusen in the subretinal space, between the retinal pigment epithelium (RPE) and Bruch’s membrane. Drusen are insoluble extracellular aggregates partially composed of cellular remnants, lipoproteins, and immune complexes. The presence of drusen is thought to incite autoimmune and inflammatory responses that damage the RPE as well as the overlying retina and photoreceptors, resulting in lesions with a depigmented, erosive appearance (geographic atrophy) on fundoscopic examination. AMD severity is classified by the number and size of drusen, the extent of geographic atrophy, and the occurrence of aberrant choroidal neovascularization. Early AMD is defined by the presence of medium-sized (63-124µm) and large drusen (>124 µm) with or without pigmentary abnormalities, while late or advanced AMD is defined by the presence of geographic atrophy or of choroidal neovascularization (neovascular AMD). Neovascular AMD represents only 10-15% of prevalent late AMD, but contributes to >80% of legal blindness (i.e. visual acuity <20/200) in AMD patients. In addition, while the clinical course of non-neovascular AMD tends to be insidious, neovascular AMD could result in rapid visual decline; proliferation of fragile, immature vessels increases the risk of hemorrhage or fluid accumulation, which in turn could lead to subretinal fibrosis or retinal detachment. While intravitreal injection of vascular endothelial grow factor (VEGF) inhibitors and laser photocoagulation may slow or limit the progression of neovascular AMD, these treatments do not reverse most of the existing damage to the macula. These findings, along with the absence of established therapies for geographic atrophy, highlight the importance of AMD prevention, particularly in an aging population.

Research suggests that oxidative stress may be involved in AMD pathogenesis. The RPE serves various supportive functions critical to the health of the retina, including phagocytosis of lipid-rich mature photoreceptor outer segments; uptake of glucose, carotenoids and lipoproteins; and maintenance of pH and fluid homeostasis. However, the close proximity of RPE to phototransduction processes renders it highly susceptible to free radical injury. Specifically, light-induced formation of reactive oxygen species and lipid peroxides could directly damage RPE cells, resulting in the accumulation of metabolic waste. Together with free radicals, these compounds could activate the complement system and cellular stress response pathways, as well as recruit inflammatory mediators such as macrophages and T-cells. Immune complex deposition then exacerbates structural injury and intensifies the inflammatory cascade, culminating in RPE dysfunction that promotes anomalous lipid deposition and angiogenesis, each distinctive characteristics of AMD progression.
**Dietary xanthophyll intake and age-related macular degeneration**

Dietary intake of the xanthophyll pigments – lutein and zeaxanthin – may play an essential role in limiting oxidative stress in the retina and the RPE.\(^4\)\(^5\)\(^6\) Xanthophylls are obtained from the diet or from supplements, and are concentrated in foods such as dark leafy greens, corn, squash, broccoli, peas and egg yolks.\(^4\)\(^5\)\(^6\) They are the predominant carotenoids found in the macula,\(^7\) and are concentrated in the outer plexiform layer overlying the photoreceptors.\(^8\) Xanthophylls are strongly absorbive of blue light, thereby reducing the amount of high-energy radiation that reaches the posterior retina and the RPE.\(^9\)

Their molecular structure also allows them to interact with free radicals via non-photochemical reactions that dissipate the transferred energy as heat (physical quenching),\(^10\) as well as to directly scavenge both singlet oxygen species\(^11\) and lipid peroxides\(^12\) via antioxidative processes (chemical quenching). Lastly, xanthophylls may have immunosuppressive and anti-inflammatory effects. Both *in vitro* studies using human cells\(^13\)\(^14\) and *in vivo* studies using animal models\(^15\)\(^16\)\(^17\) have demonstrated that lutein and zeaxanthin supplementation may influence the expression of inflammation-related genes associated with AMD, including complement factor H (CFH),\(^18\)\(^19\)\(^20\) interleukin 8 (IL-8),\(^21\)\(^22\)\(^23\) monocyte chemoattractant protein 1 (MCP-1),\(^24\)\(^25\)\(^26\) and nuclear factor kappa-light-chain enhancer of B cells (NF-κB).\(^27\) Lutein supplementation has been shown to decrease circulating levels of complement factors and inflammatory cytokines in humans.\(^28\)\(^29\)\(^30\) Furthermore, a dietary intervention that increases plasma xanthophylls has been found to decrease lipid peroxidation in humans.\(^31\)

Randomized trials of lutein and zeaxanthin supplementation have been shown to: 1) increase average xanthophyll retinal concentrations as measured by macular pigment optical density (MPOD),\(^32\)\(^33\)\(^34\) 2) preserve visual acuity in early AMD,\(^35\)\(^36\)\(^37\) and 3) decrease progression to advanced AMD when replacing beta carotene in individuals receiving a high-dose antioxidant regimen as well as in study participants who had low dietary intake at the start of the trial.\(^38\) No clinical trial has investigated whether xanthophyll supplementation decreases risk of early AMD. Prospective cohorts have shown that xanthophyll intake is positively related to MPOD,\(^39\)\(^40\) and may be protective against neovascular AMD.\(^41\)\(^42\) While only one study has found a relationship between xanthophyll intake and advanced AMD (both choroidal neovascularization and central geographic atrophy),\(^43\) the pooled estimate from a meta-analysis of five previous studies suggest a significant negative association.\(^44\) With the exception of the Blue Mountains Eye Study,\(^45\) xanthophyll intake did not appear to influence the incidence of early AMD in various cohorts (i.e. Rotterdam Study, Beaver Dam Eye Study, Nurses’ Health Study, and Health Professionals Study).\(^46\)\(^47\)\(^48\)\(^49\) However, a cohort that initially found no association between xanthophyll intake and prevalence of early/intermediate AMD in postmenopausal women observed a significant inverse relationship after restricting the analyses to younger participants with stable dietary intake and without a history of chronic diseases (e.g. cardiovascular disease, diabetes and hypertension) that frequently lead to dietary changes.\(^50\) In addition, there is evidence to suggest that xanthophyll intake may be associated with the development of retinal pigmentary abnormalities, a hallmark of early AMD, though the results have also been mixed.\(^51\)\(^52\)\(^53\)

**Genetic susceptibility as a potential effect modifier**

Inconsistent results between xanthophyll intake and early AMD could reflect the presence of unmeasured effect modifiers. In particular, differential genetic susceptibility could modify the relationship between dietary xanthophyll intake and early AMD. Though the subject remains under vigorous debate, a randomized trial showed that the effects of antioxidant supplementation on AMD progression may vary by CFH and ARMS2 genotype.\(^54\)\(^55\)\(^56\) Pooled analysis of the Blue Mountains Eye Study and Rotterdam Study further demonstrated that increased xanthophyll intake reduced incidence of early AMD only among individuals at higher genetic risk, as determined by the genotypes of the CFH rs1061170 and ARMS2 rs10490924 polymorphisms.\(^57\) Interestingly, homozygosity at these two loci has
also been linked to lower MPOD, independent of serum xanthophyll levels.\textsuperscript{94} Whether CFH and ARMS2 interact with xanthophyll intake to influence early AMD risk thus warrants further consideration.

**High-density lipoprotein metabolism as a potential effect modifier**

Perturbations in lipid metabolism have been implicated in AMD pathogenesis. Lipid deposits lining Bruch’s membrane precede the formation of drusen,\textsuperscript{95} and occupy a significant fraction (37-44\%) of drusen volume.\textsuperscript{96} Low-density lipoprotein (LDL) is responsible for the majority of cholesterol delivery to the retina.\textsuperscript{97} However, high-density lipoprotein (HDL) also participates in cholesterol trafficking to the retina,\textsuperscript{97} and is the predominant carrier of circulating xanthophylls.\textsuperscript{114,115} Research further suggests that HDL has antioxidant and anti-inflammatory properties,\textsuperscript{98,99} and may be involved in complement regulation.\textsuperscript{100}

While some studies have found inverse associations between total HDL concentration (HDL-c) and AMD,\textsuperscript{101-104} others have reported positive\textsuperscript{105-112} or null\textsuperscript{113-118} associations. In addition, though HDL-c has been positively associated with plasma xanthophyll levels,\textsuperscript{119-124} most studies have found HDL-c to be unrelated to MPOD.\textsuperscript{119-121,125} At the same time, some genetic polymorphisms in the HDL pathway may decrease AMD risk and/or increase plasma xanthophyll levels without influencing HDL-c.\textsuperscript{126,127} Apolipoprotein A1 (apoA1) is the major protein component of HDL, and mediates reverse cholesterol transport from macrophage-derived foam cells in the periphery back to the liver.\textsuperscript{100} Additionally, a recent \textit{in vitro} study showed that ApoA1 may facilitate intestinal uptake of xanthophylls by HDL.\textsuperscript{128} Measurement of serum ApoA1 is more reliable than that of HDL-c, as it is not influenced by concomitant triglyceride levels.\textsuperscript{129} Serum ApoA1 concentrations have further been inversely related to cardiovascular disease,\textsuperscript{130-132} diabetes,\textsuperscript{133-136} and chronic kidney disease.\textsuperscript{137,138} Interestingly, larger and lighter HDL particles (HDL2 subfraction) tend to carry higher concentrations of xanthophylls and apoAI\textsuperscript{129} than smaller and denser HDL particles (HDL3 subfraction).\textsuperscript{139} HDL2 concentration has further been inversely related to cardiovascular disease\textsuperscript{140} and diabetes mellitus,\textsuperscript{141} systemic diseases associated with higher risk for AMD.\textsuperscript{117,142-144} Collectively, these findings indicate that serum HDL-c and other serum biomarkers of HDL metabolism may help define HDL metabolism, that systemic and intraretinal xanthophyll transport by HDL are related but distinct processes, or that HDL may influence AMD risk via non-lipid factors.

\textit{More work is needed to better understand the association between xanthophyll intake and early AMD. The interactive effects of CFH rs1061170, ARMS2 rs10490924, and dietary xanthophyll intake on AMD have not been thoroughly explored, and to our knowledge, previous studies have not examined the association between xanthophyll intake and early AMD by HDL-c or other serum biomarkers of HDL metabolism:}

5. **Main Hypothesis/Study Questions:**

The aims of our study are two-fold:

**Main Study Question 1:**
Is there an association between dietary xanthophyll (lutein and zeaxanthin) intake, assessed at visit 1 (1987-1989) and prevalence of early AMD, assessed at visit 3 (1993-1995)?

**Main Study Question Hypothesis:**
We hypothesize that participants with higher compared to lower xanthophyll intake will have lower odds for early AMD.

**Exploratory Study Question 1:**
Is the association between dietary xanthophyll intake and early AMD modified by CFH rs1061170 and ARMS2 rs10490924?

**Exploratory Study Question 1 Hypothesis:**
We hypothesize that higher xanthophyll intake will be associated with lower odds of early AMD only among participants at high genetic risk (≥2 alleles of either CFH rs1061170 C or ARMS2 rs10490924 T).

**Exploratory Study Question 2:**
Is the association between dietary xanthophyll intake and AMD modified by measures of HDL cholesterol levels (HDL-c) and other serum biomarkers of HDL metabolism (HDL2 and apolA1)?

**Exploratory Study Question 2 Hypothesis:**
We hypothesize that higher xanthophyll intake will be associated with lower odds of early AMD only among participants with high HDL-c, HDL2 or apolipoprotein A1.

**Additional Study Questions**
We will evaluate whether the pattern of results varies by race.

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 AMD was determined via fundus photographs taken at visit 3 (1993-1995), using a non-mydriatic automatically focusing camera (Canon CR-45UAF). Patients were asked to sit in a darkened room for 5 minutes, after which the camera was centered on the region between the optic disc and the fovea, and a nonstereoscopic 45-degree retinal image was obtained of one eye without the use of pharmacologic dilation. Fundus photographs were evaluated by a masked grader at the University of Wisconsin Fundus Photograph Reading Center for the presence of soft drusen >63µm in diameter, RPE and retinal hypo-/hyperpigmentation, geographic atrophy, and neovascular AMD. Gradable fundus photographs were obtained from 11,532 of 12,887 eligible participants (89.5%). Of these, 596 (5.2%) exhibited signs of any AMD, 581 (5.0%) showed signs of early AMD, and 15 (0.13%) showed signs of advanced AMD. Given the low prevalence of advanced AMD, the primary endpoint variable will be defined as the presence of early AMD.

**Assessment of dietary xanthophyll intake**
A 66-item food frequency questionnaire (FFQ) completed at visit 1 (1987-89) will be used to estimate dietary intake of lutein and zeaxanthin. This instrument was modified from a version developed by Willet et al, and its validity and reliability has been demonstrated. Dietary xanthophyll intake will be adjusted for total energy intake. Participants with implausible total energy intake or with ≥10 (≥15%) missing values on the FFQ will be excluded. Dietary supplements of these compounds were not available during the time of the study. Dietary xanthophyll intake will be operationalized as a categorical variable (e.g. greater or less than race-median combined lutein and zeaxanthin intake per the results of
previous National Health and Nutrition Examination Surveys,\textsuperscript{88,149} and by quintiles). We will also examine associations between continuous concentrations of xanthophyll intake and prevalent early AMD. FFQ data gathered at visit 3 (1993-95) will also be used to explore associations between AMD and xanthophyll intake at visit 3, using the averaging intake from visits 1 and 3, and among those whose xanthophyll intake changed minimally from visits 1 and 3 (defined using change between quintiles at both time points).

**Genetic data:** Genotyping of single nucleotide polymorphisms (SNPs) in ARIC was completed using the Affymetrix Genome-Wide Human SNP Array 6.0.\textsuperscript{150} Data are available on two high risk SNPs (\textit{CFH} Y402H [rs1061170] and \textit{ARMS2} A69S [rs10490924]) shown to be associated with increased risk of early AMD.\textsuperscript{151} We will use this data to explore whether either variant confounds or modifies the association between xanthophyll intake and AMD.

**Assessment of serum biomarkers of HDL-metabolism**

Participants were asked to fast for $\geq 12$ hours prior to the clinical examination. Blood was drawn from the antecubital vein into tubes containing EDTA, which were then fractionated via centrifugation at $3000g$ and $4^\circ C$ for 10 minutes. Plasma samples were stored at $-70^\circ C$ until analysis at the ARIC Central Lipid Laboratory. Total cholesterol and triglycerides concentrations were assayed using the cholesterol oxidase-4-aminophenazone reaction scheme. HDL was then separated into subfractions and quantified using the Warnick dual-precipitation method. Apolipoprotein A1 concentration was determined via radioimmunoassay.\textsuperscript{152} Measurements of HDL biomarkers from visit 1, converted to International System of Units values (mmol/L), will be considered for these analyses.

**Proposed analysis**

The distribution of participant characteristics and other risk factors according to dietary xanthophyll intake (quintiles), AMD status (none vs. early), and the presence of pathologic stigmata of early AMD (i.e. soft drusen, retinal pigmentary abnormalities) will be examined using chi-square test, t-test and analysis of variance. Bivariate relationships among these variables will also be explored using Pearson product-moment correlation, point-biserial correlation, or chi-square test. Tables summarizing these descriptive statistics will be presented.

Multivariate logistic regression will be used to evaluate the association between quintiles of dietary xanthophyll intake and AMD status. Crude, age-adjusted and multivariate-adjusted odds ratios, 95% confidence intervals, and p-values for trend analyses (using quintile medians) will be reported. Potential confounders will be identified using previous studies or the change-in-estimate method ($\geq 10\%$ change in the OR). Identified confounders will be adjusted for in subsequent multivariate analyses. Effect modification by high risk genotype and HDL cholesterol levels (HDL-c) and other HDL-related biomarkers will be assessed via interpretation of the multiplicative interaction terms, synergy indices\textsuperscript{153} and stratified analyses. Specifically, we will investigate interaction by adding multiplicative interaction terms (e.g., xanthophyll intake $\times$ HDL-related variable) to the regression model. A p-value for interaction of $<0.10$ will be considered statistically significant. All HDL-related biomarkers will be operationalized as continuous variables and as categorical variables based on existing guidelines or recommendations for prevention of atherogenesis.\textsuperscript{154} To our knowledge, no threshold values have been established for HDL2.

**Limitations and possible solutions**

The proposed study has several limitations. First, the use of prevalent AMD as an outcome precludes inference of causality. It is impossible to determine whether maculopathy was already present at
baseline, and whether the reported dietary patterns reflect long-term intake. However, previous research using a subset of the ARIC cohort suggests that responses on the FFQ were reliable across the 3-year interval of interest.\textsuperscript{145} Since secondary analyses of the AREDS2 randomized trial showed significant effects of xanthophyll supplementation after a median follow-up of 4.9 years,\textsuperscript{77} it is plausible that differential xanthophyll intake during the ARIC study may have influenced AMD status. In this study, baseline/visit 1 (1987-1989) and visit 3 (1993-1995) FFQ responses will be used to assess changes in and stability of dietary behaviors. Change in xanthophyll intake will be tested as a potential confounder in multivariate models. All analyses will also be repeated after excluding participants reporting a significant change (e.g. >1 quintile) in dietary xanthophyll intake.

Second, in this sample, prevalence of advanced AMD was low (0.13%), even among persons over the age of 65 (0.4%). Thus, analyses may be limited to using early AMD or any AMD as the outcome of interest. Moreover, only 12,887 of the 26,427 participants (82% of the survivors) recruited at baseline (1987-1989) returned for Visit 3 (1993-1995). Of these individuals, 1,317 had ungradable fundus photographs, and may have been at greater risk of AMD (i.e. older, more likely to have diabetes mellitus, and more likely to have evidence of CVD on magnetic resonance imaging).\textsuperscript{145} Taken together with the high rate of non-participation, conclusions drawn from this study may be susceptible to selection bias. Multivariate analyses will therefore be interpreted with and without adjustment for the propensity score, or the conditional probability of an outcome given a set of observed confounders.\textsuperscript{155}

The propensity score will be constructed using covariates that differed in distribution by fundus photograph status (gradable vs. ungradable).

7.a. Will the data be used for non-CVD analysis in this manuscript? \textbf{X} Yes \textbf{N} No

b. If Yes, is the author aware that the file ICTDER03 must be used to exclude persons with a value RES\textunderscore OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES\textunderscore DNA = “CVD Research” would be used? \textbf{X} Yes \textbf{N} 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? \textbf{Y} Yes \textbf{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\textunderscore DNA = “No use/storage DNA”? \textbf{X} Yes \textbf{N} 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

\textbf{X} Yes \textbf{N} 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 involve other work on age-related macular degeneration and would involve Drs. Ronald and Barbara Klein, both of whom 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.

**Works Cited**


74. Murray IJ, Makridaki M, van der Veen RLP, Carden D, Parry NRA, Berendschot TTJM. Lutein supplementation over a one-year period in early AMD might have a mild beneficial effect on visual acuity: the CLEAR study. Invest Ophth Vis Sci 2013;54:1781-8.


