# Research Protocol

**Carotenoids and Age-related Eye Disease in the Women's Health Initiative (CAREDS)**

**Principal Investigator: Julie Mares-Perlman, PhD**

**University of Wisconsin - Madison**
**Department of Ophthalmology & Visual Sciences**

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**Participating Sites: University of Wisconsin - Madison, University of Iowa - Iowa City and Kaiser Center for Health Research - Portland**

## Background

Age-related eye diseases of the lens and retina are common in older Americans.[1-3] For example, approximately 55%-85% of Americans between the ages of 75 and 85 years have been estimated to either have cataracts or have undergone cataract surgery[3,4] and 3% to 7% have late stages of macular degeneration.[1,2] In the next 50 years, when the number of people over the age of 75 years is expected to triple,[5] these conditions will be even more common. These age-related conditions currently impose a large burden in health care costs[6] and this burden will increase as the population ages.[7] There is some evidence to suggest that certain plant pigments (the xanthophyll carotenoids, lutein and its structural isomer zeaxanthin) that are present in the foods we eat may protect against age-related macular degeneration (ARMD) and cataract.

Carotenoids have been hypothesized to protect against other age-related chronic conditions such as cardiovascular disease and cancers.[8,9] There is evidence that the absorption of carotenoids by the gastrointestinal tract and the uptake into tissues is variable across

individuals,[10] but there is little knowledge about the specific factors that influence uptake.[11] Tissue concentrations of carotenoids cannot be assessed non-invasively, except in the eye where levels of macular carotenoids can be determined by a novel technique using pyschophysical flicker photometry[12-14].

Despite popular interest in the possibility that the xanthophyll carotenoid lutein, and its structural isomer zeaxanthin, may protect against the onset or progression of age-related macular degeneration, data to support this relationship is insufficient. Data are also accumulating to support a possible protective affect of diet xanthophylls on nuclear cataract. Observational studies that reflect long term relationships of intake of these xanthophyll carotenoids to their accumulation in the retina and the occurrence of these conditions in human populations are needed.

## Purpose and Specific Aims

This investigation augments information being collected in the National Institutes of Health sponsored Women's Health Initiative - Observational Study (WHI-OS) in order to identify specific dietary protective factors for the most common, costly and debilitating age-related eye conditions: age-related macular degeneration (ARMD) and nuclear cataract. This ancillary study is designed to investigate the relationships of xanthophyll carotenoid pigments in the diet and blood to those in the macula and to the prevalence of early age-related maculopathy (ARM) and nuclear cataract. The investigators hypothesize that women who have consistently low, compared with high, long-term dietary intakes of xanthophylls will have lower macular pigment density, higher prevalence of specific early macular abnormalities associated with ARM, and higher prevalence of nuclear cataract, even after adjusting for other lifestyle and diet attributes that may be correlated both with high xanthophylls diets and these eye diseases.

A second purpose of this study is to determine the physiologic and lifestyle attributes that influence the relationship between the intake of xanthophyll carotenoids and their presence in blood and in the ocular macula. This information may also provide insights regarding the determinants of tissue concentrations of carotenoids and can be applied towards a greater understanding of the relationships between carotenoids and the chronic diseases of aging.

Specific aims of this investigation are to:

1. Determine whether women in the WHI-OS selected from three sites who have sustained dietary levels of lutein and zeaxanthin in the lowest, compared to highest, quintile have:
   a. lower macular pigment density of xanthophylls,
   b. higher prevalence of ARM and specific macular lesions which increase the risk for developing late ARMD:
      - large, soft, indistinct drusen and
      - pigmentary abnormalities, and
   c. more severe nuclear sclerosis of the lens;

and to determine whether these relationships are maintained after adjusting for other lifestyle, medical and diet attributes that may be correlated with high xanthophyll diets and these eye conditions.

2. Identify dietary, lifestyle, health history and physiologic determinants of macular pigment density in women.

## Methods

The study will be conducted in a cohort of women who are enrolled in the Women's Health Initiative - Observational Study (WHI-OS) at three sites located in Madison, WI (Coordinating Center), Portland, OR and Iowa City, IA. This cohort is ideal for this investigation because it includes women at the age of high risk for early ARM. In addition, this study will benefit from the availability of existing blood samples and a wealth of health and dietary information previously collected for the WHI-OS.

The Women's Health Initiative (WHI) was initiated in 1993 at 40 clinical centers across the United States to investigate prevention and control of the most common causes of morbidity and mortality among postmenopausal women. The cohort of women enrolled in the WHI-OS are being followed to study the relationship between risk factors and disease over the ten-year course of the study. They have completed initial examinations at baseline and three years later and data has been collected regarding diet, lifestyle practices and medical history.
Participants in WHI-OS whose xanthophyll intake at study entry were in highest and lowest quintiles will be identified by the coordinating center of the WHI (Fred Hutchinson Cancer

Research Center, FHCRC). The number of WHI-OS participants that is expected to be categorized in high and low quintiles for dietary xanthophylls is 2713 (across all three centers). Based on high retention rates in the WHI-OS, it is expected that 2441 women may be enrolled in this study. A list of potential participants will be sent from FHCRC to the participating center's WHI Principal Investigator.

Each participating WHI site will develop a recruitment plan to facilitate timely enrollment into the study. The recruitment plan will include an invitation letter and study fact sheet mailed to potential participants and follow-up phone calls to ascertain level of interest. The WHI site staff will phone potential participants and answer any preliminary questions that the participant may have about the study. Women who agree to participate will be scheduled for an eye exam and will be mailed a study packet containing a consent form and study questionnaires.

Each participating WHI site will provide the CAREDS Coordinating Center (at the University of Wisconsin - Madison) with monthly recruitment reports. Reports will include the number of participants contacted and number of participants scheduled for eye exams. Potential participants will not be identified by study ID number or name in these reports. Women who decline participation will be recorded (not identified) for informational purposes only to rule out any potential bias due to nonparticipation.

It is expected that enrollment at the University of Wisconsin - Madison will begin in January 2001 and will be completed at all three centers within 18 months.

## Procedures

Participants will be considered enrolled in the study on the date that the consent form is signed and the eye exam is performed.

### *Study Questionnaires*

The following questionnaires will be completed by each participant: Food Frequency Questionnaire (FFQ), Food Questionnaire: Past Diet, Sunlight Exposure Questionnaire, and Lifestyle Update and Family History Questionnaire. It is anticipated that these questionnaires will take about 60 minutes to complete. The participant will be asked to complete these questionnaires prior to coming to the clinic for the eye exam.

### *Eye Exam, Photography and Processing*

Written informed consent will be obtained prior to performing the eye exam. The clinical coordinator will interview the participant regarding their ocular health prior to the exam. In addition, the clinical coordinator will review the study questionnaires for completeness. If any of the questionnaires are incomplete, the participant will be asked to complete them before the eye exam.

1. Visual acuity will be measured in each eye using the standardized Early Treatment Diabetic Retinopathy Study protocol[15] as modified in the Age-Related Eye Disease Study (AREDS)[16.] Using the best corrected visual acuity, the macular pigment density will be measured in both eyes by a non-invasive psychophysical test using heterochromatic flicker photometry.[12-14]

2. Both eyes will be examined with slit lamp biomicroscopy and the anterior chamber depth will be evaluated. Participants with no new ocular symptoms will not be seen by an ophthalmologist unless the examiner considers that the anterior chamber angle may be occludable. In this case, an ophthalmologist will evaluate the participant prior to dilation of the pupils. If there is a risk of angle closure, the participant's eyes will not be dilated.

3. If there is no risk of angle closure, both eyes will be pharmacologically dilated. After dilation of the pupils, a single nonstereoscopic photograph will be taken of each eye with a modified Topcon slit lamp camera. A Zeiss fundus camera will then be used to take non-simultaneous, $30^{o}$ stereoscopic pairs of photographs of the fundus according to the AREDS protocol.

All photos (slides) will be mailed to the Reading Center at the University of Wisconsin within one week of examination. Preliminary grading will be accomplished within one week of receipt to identify: 1) any clinically relevant conditions requiring participant/physician notification and 2) any deviations in photographic quality. Photos will be reviewed for nuclear sclerosis, nuclear color, and any signs of relevant conditions or abnormalities. Detailed grading of the lens and retina will occur following protocols established for the AREDS study[16-17].

If any lesions which threaten vision or life are noted during preliminary grading, the Reading Center will contact the CAREDS Coordinating Center, who will in turn immediately contact the WHI Clinical Site. The WHI Clinical Site will immediately notify the participant. The CAREDS Coordinating Center Staff will not directly contact the study participants.

### *Follow-up*

Participants who complete the eye exam will be mailed feedback letters from the CAREDS Coordinating Center (University of Wisconsin - Madison) following final grading of photographs (within 4-6 weeks of exam). Feedback letters will thank the subject for participation and will contain eye exam results. If the participant wishes, eye exam results will be also be sent to their primary physician and/or ophthalmologist. Copies of participant feedback letters will also be sent to each participating WHI site.

### *Blood Analyses*

Serum samples were obtained from WHI-OS participants in baseline examinations (1994-98) and have been stored at -80 degrees centigrade. Samples will be shipped to a central reference laboratory for analysis of serum carotenoids and tocopherols by reverse-phase high performance liquid chromatography. Total cholesterol will be analyzed (if has not been previously determined) to evaluate the effect of adjusting for this value.

Serum levels of lutein and zeaxanthin will permit the identification of women who are possible extreme non-responders to dietary lutein. Possible non-responders among women with high dietary intakes will be defined as women who have serum values less than the 20th percentile in a sample of WHI participants (approximately 140 ng/ml based on preliminary analysis). Serum levels of other carotenoids and tocopherols which are concurrently available in assays that quantitate serum lutein will be available to use to assess possible confounding by these compounds which have also been hypothesized to be related to ARM.

Participants will not be informed of the results of the serum analyses. Samples will be discarded after the study is completed.

### *Currently Available Data*

Extensive information regarding demographic attributes, lifestyle, medical history and diet, as well as anthropometric and physical measurements were collected from participants on entry to WHI-OS. In addition, WHI-OS participants complete yearly questionnaires by mail and participate in a second visit at Year 03. The types of data available from previously administered questionnaires are summarized in the Table 1. Data pertaining to current smoking habits, alcohol consumption and hormone use will be updated for this study.

Table 1 - Summary of Data Available for WHI-OS Study Participants

1. Age, Education, Employment History, Income, Race
   Lifestyle
2. Alcohol intake: history at entry and at three-year follow-up
3. Smoking history: smoking at entry and at three-year follow-up
4. Supplement use: current and duration
5. Special diets

Anthropometric and Physical Measurements

1. Weight: at birth, age 18, 35 and 50
2. Height
3. Weight loss history
4. Waist/Hip measurement

Medical History

1. Hormone use
2. Current medications
3. History of cancer, diabetes, cerebrovascular disease, cataract, glaucoma, coronary heart disease, arthritis, hypertension, peripheral arterial disease, intestinal polyps, fractures
4. Reproductive history
5. Family history of diabetes, heart attack, stroke, cancer, fractures
6. Three-year self-reported incidence of cataract, macular degeneration, and other misc. medical conditions

Diet

1. Food frequency questionnaire at entry (into WHI-OS)

## Risks

The risks involved in the measurement of macular pigment density and eye photography are negligible. There is a slight risk of mild local allergic reaction from drops used to dilate the pupils, which will be minimized by asking the participant about any prior episodes following use of drops. This is some risk of angle closure glaucoma, particularly in those with shallow anterior chambers. Unless there is a history of allergy or unless the angle is found to be potentially occludable on slit lamp examination, pupils will be pharmacologically dilated. All persons will be advised of possible side affects and will be asked to call the eye clinic promptly if they experience ocular pain, headache, or blurring of vision greater or more prolonged than expected from pupillary dilation. Individuals with shallow anterior chambers will not be dilated (photographs will still be taken). If the anterior chamber angle has closed, appropriate management will be instituted.

If a medical problem is recognized at the time of the examination that requires prompt attention, the physician in charge of the study at individual sites or designated substitute physician will be notified immediately. In addition, the participant's primary physician will be notified.

WHI labels with study ID numbers will be used on questionnaires and forms that will be stored at the University of Wisconsin CAREDS Coordinating Center. Unique identifiers but not names will be used on slide shipments to the Reading Center. The CAREDS Coordinating Center will keep a separate database of participants' addresses. Access to this database will be limited and this database will not be linked to participants' study ID number and/or study results. Data to be published will deal only with groups of participants and no participant will be identified by name.

## Benefits

*Participant:* A brief explanation of age-related ocular diseases will be given to each participant

and results of the eye examination communicated to the participant. Those persons with severe disease, for example age-related macular degeneration for which photocoagulation treatment may be indicated and who are not aware of this situation, may benefit from being made aware of the need for ophthalmic consultation and possible treatment.

*Society:* This study utilizes new advances in novel techniques to reliably detect levels of xanthophyll in the retina and a unique opportunity to extend investigations in the WHI to evaluate possible means of preventing or delaying common, costly and debilitating eye conditions. The study will provide an estimate of the degree to which dietary choices involving xanthophylls could impact the density of these pigments in the retina and risk for eye disease. These estimates may help determine if there is justification for clinical trials to evaluate the impact of dietary lutein and/or zeaxanthin supplements on the development or progression of ARM and nuclear cataract.

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