1 INTRODUCTION AND BACKGROUND

The purpose of this research study was to evaluate whether hormone replacement therapy in women would prevent or slow the progression of age-related maculopathy (ARM). ARM is a leading cause of permanent visual impairment among Americans aged 65 and older. \(^1\) It is gradually progressive, often over a period of more than five years. Estimates of prevalence range widely across studies, depending on the age of the population, geographical location and method of ascertainment. Table 1 summarizes the studies that have reported prevalence and their estimates. Studies for this estimate were selected from population-based studies with relatively large sample sizes. Methods of ascertaining ARM were based on widely accepted standardized approaches such as the Wisconsin ARM Grading System. Studies that were based on convenience samples or nursing home populations were not included.

<table>
<thead>
<tr>
<th>Author/study</th>
<th>Geography</th>
<th>Age</th>
<th>Prevalence</th>
<th>ARM Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robman, 1999</td>
<td>Australia</td>
<td>55-80</td>
<td>0.5%</td>
<td>Any ARM</td>
</tr>
<tr>
<td>Klaver, 1999</td>
<td>Netherlands</td>
<td>75+</td>
<td>4.5%</td>
<td>Late ARM</td>
</tr>
<tr>
<td>Klein, 1999</td>
<td>US</td>
<td>48-72</td>
<td>5.6%</td>
<td>Any ARM</td>
</tr>
<tr>
<td>Klein, 1999</td>
<td>US/Australia</td>
<td>&lt;86</td>
<td>1.2-1.4%</td>
<td>Late ARM</td>
</tr>
<tr>
<td>Friedman, 1999</td>
<td>Baltimore</td>
<td>40+</td>
<td>2.1%</td>
<td>ARM</td>
</tr>
<tr>
<td>Klein, 1992</td>
<td>US</td>
<td>75+</td>
<td>5.2%/75+</td>
<td>(1) Exudative</td>
</tr>
<tr>
<td>Pagliarini, 1997</td>
<td>Italy</td>
<td>70+</td>
<td>1.1%</td>
<td>(2) geographic</td>
</tr>
<tr>
<td>Cruickshanks, 1997</td>
<td>Wisconsin, Colorado</td>
<td>43-74, 21-74</td>
<td>14.3%</td>
<td>Any ARM</td>
</tr>
<tr>
<td>Reidy, 1998</td>
<td>UK</td>
<td>65+</td>
<td>8%</td>
<td>Vision impairing ARM</td>
</tr>
<tr>
<td>Klein, 1999</td>
<td>US/white</td>
<td>40+</td>
<td>9.4%</td>
<td>Any ARM</td>
</tr>
<tr>
<td></td>
<td>US/Mexican</td>
<td></td>
<td>7.1%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>US/African Am.</td>
<td></td>
<td>7.4%</td>
<td></td>
</tr>
<tr>
<td>Dickinson, 1997</td>
<td>UK</td>
<td>77-90</td>
<td>3.2-3.8%</td>
<td>Exudative, geographic</td>
</tr>
<tr>
<td>Laatikainen, 1995</td>
<td>Finland</td>
<td>70+</td>
<td>4.7%</td>
<td>ARM</td>
</tr>
<tr>
<td>Delcourt, 1999</td>
<td>France</td>
<td>70-79</td>
<td>1.5%</td>
<td>(1) Exudative,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>15.2%</td>
<td>geographic, (2) soft distinct drusen</td>
</tr>
</tbody>
</table>

Only two studies have reported gender differences: the Beaver Dam study reported that women 75 years of age and older have a higher prevalence of exudative macular degeneration than men (7% versus 3%).\(^2\) Delcourt reported an approximately equal prevalence of late ARM in men (1.7%) and women (1.5%) aged 70-79\(^3\). Risk factors associated with ARM include hereditary factors; cardiovascular disease (CVD) and CVD risk factors; diabetes and glycemic status; smoking; chronic exposure to light; dietary and blood levels of zinc or antioxidant vitamins; and the use of post-menopausal estrogens. At this time, there was neither a preventive approach nor cure for ARM. Currently, treatment is limited to laser photocoagulation, which has a statistically significant, though quantitatively very limited, effect in reducing the progression of visual loss in highly selected cases of neovascular ARM\(^4\). Some forms of ARM may respond to treatment with high dose vitamin therapy or other dietary interventions\(^5\). There may be ethnic differences in ARM: one report describe a lower occurrence of ARM in blacks\(^6\). However, others report no ethnic differences after age adjustment\(^7\). Dietary antioxidants have been examined in relation to the risk of ARM: several observational studies have reported that vitamins C, E and carotenoids...
are associated with reduced risks of ARM. The Blue Mountains Eye Disease Study did not find an association between alpha tocopherol or beta carotene and ARM.

**Estrogen Replacement Therapy.** Several studies have reported a higher occurrence of ARM in women compared to men. Vingerling reported that early menopause (<age 45) was associated with a 90% increase in risk of ARM. This was highest in those with surgical menopause involving an oophorectomy (RR, 3.8). Two published observational studies have addressed the association between ARM and estrogen replacement therapy (HRT). The Eye Disease Case Control Study found the risk of neovascular ARM was reduced by 70% in women who were current users of HRT and by 40% in women who were former users. Adjustment for covariates did not affect these associations. A report from the Beaver Dam Study based on cross-sectional data also suggested a protective effect of HRT on ARM. Another study found a protective association for early ARM with increased years from menarche to menopause, suggesting a protective effect with longer exposure to endogenous estrogen. To date, there have been no clinical trials conducted to examine this protective association between estrogenic hormones and ARM. Possible mechanisms by which estrogen may influence the risk of ARM include its effects on vascular factors which have been variously linked to ARM. The presence of estrogen receptors in the retina and retinal pigment epithelium has suggested that estrogen could play a role in ocular diseases. Vingerling has reported a 2.5-4.7 fold increased risk of ARM associated with atherosclerosis in several cardiovascular beds. Estrogen affects microvascular circulation through its influence on endothelial function, vascular reactivity, and a range of hemostatic and coagulation factors. If ARM is linked to or influenced by vascular factors, estrogen could play a role in preventing this disease. Genetic factors also play an important role in maculopathy and could potentially modify any effects of estrogen on the risk of maculopathy. The association between apoE lipoprotein E and ARM was examined in the Rotterdam study which reported a 57% reduced risk of ARM in those with ApoE4. Work by Souied found a lower frequency of E4 in those with the exudative form of ARM. Estrogen is widely recognized as an antioxidant: the antioxidant activity of estrogens may be another mechanism by which this compound could reduce the risk of ARM in women. Since the effects of estrogen may be modulated by ApoE, genetic factors may also modify the relationship between HRT and ARM.

## 2 OBJECTIVES

The primary goal was to evaluate the efficacy of Premarin® (conjugated equine estrogen (CEE)) and Prempro™ (conjugated equine estrogen plus medroxyprogesterone (CEE + P)) versus placebo in slowing the progression of ARM as measured by a systematic grading of stereoscopic retinal fundus photographs.

## 3 STUDY DESIGN

The Women’s Health Initiative Sight Exam study (WHI-SE) was an ancillary study to the Women’s Health Initiative Hormone Replacement Therapy Clinical Trial (WHI HRT CT), and enrolled 4,347 women who were in the HRT of the study. Data collection was completed. The Women’s Health Initiative (WHI) was launched in 1991 by the National Institute of Health (NIH). WHI consisted of several clinical trials and an observational study and included generally healthy postmenopausal women. WHI HRT CT was one of the WHI clinical trials and was a randomized, multi-center (21 sites), double-blinded clinical trial of conjugated equine estrogen, 0.625 mg per day (CEE) for women without a uterus or conjugated equine estrogen, 0.625 mg per day plus medroxyprogesterone, 2.5 mg per day (CEE+P) for women with a uterus, versus placebo. CEE or CEE+P was assigned in a 1:1 ratio to placebo.
WHI HRT CT participants from 21 centers were recruited to participate in WHI-SE between April 2000 and June 2002, an average of 5.1 (median, 5.0; range, 1-10) years after randomization. Data on risk factors for eye disease or on eye disease diagnoses were not generally collected as part of the main study, nor were relevant biochemical or genetic measures available to the WHI-SE. The WHI-SE study added fundus photography for assessment of age-related maculopathy to the main study. In addition, data about visual acuity and self-reported visual functioning, eye disease history, risk factor exposures were collected. Blood for biochemical analysis and assessment of genetic factors were collected and banked for future analysis when additional funding becomes available. None of these data were collected as part of the main WHI study. WHI-SE participants underwent an eye exam and fundus photography session, completed at a brief questionnaire on eye disease and treatments, and a one-time venipuncture for blood/serum was collected. Study participants were contacted by mail or telephone annually at the anniversary of their original eye exam to respond to questions about new diagnoses of maculopathy and other eye diseases and surgeries, such as cataract surgery. When a new diagnosis of maculopathy was reported, the WHI-SE Coordinating Center obtained verification by mail from the diagnosing physician. Thus, there were two complete sets of fundus photography obtained by the WHI local clinical center and four annual contacts by telephone conducted by the WHI-SE Coordinating Center. Determination of incidence was based on the fundus photographs and verified ARM diagnoses in interim years. Data collection during interim years is necessary to obtain data on diagnoses of ARM that might occur in women who die or drop out of the study and are not seen at the second evaluation. The total duration of the study was 7 years and participants were followed for 4 years, on average. After close out of the main WHI study, HRT information was obtained and added to the study data to evaluate the association of HRT with age-related maculopathy (ARM).

WHI-SE was originally designed to include both a cross-sectional eye examination and a second eye examination to capture ARM incidence, but the sponsor canceled the study in the wake of the WHI HRT CT results so the second examination could not be done. Thus, WHI-SE was a cross-sectional study.

4 SELECTION OF PATIENTS
Since the study population was drawn from the WHI HRT Clinical Trial, patients were already included or excluded based on those criteria. Refer to the WHI HRT Clinical Trial Study protocol for more detail.

4.1 Inclusion Criteria
1. Women aged 65 years or older currently enrolled in the WHI HRT Clinical Trial.
2. Speak and read English or Spanish, agree to complete study questionnaires and two eye examinations with fundus photography, and sign the informed consent document
3. Patient has at least one eye that can be dilated for the purpose of retinal fundus photography

4.2 Exclusion Criteria
1. Allergies to dilating eye drops
2. Other known contraindications for the administration of dilating eye drops
3. Any reason that the participant cannot be subjected to retinal fundus photography
5 STUDY METHODS

5.1 Schedule of Visits

5.1.1 Screening and Baseline Evaluation (Visit 1 and 2, Weeks –12 to 0)
Visit 1. Review of protocol with subject, screen for contraindications (allergies to dilating drops or neither eye able to be dilated) and consent to participate obtained prior to study entry. Patient completed a questionnaire on visual function, history of light exposure, vitamin, and medication usage. Questionnaire was returned to WHI-SE Coordinating Center after review by WHI CC staff. Patient had a blood draw of 32 ml of blood.

Visit 2. Patient was scheduled for an eye exam and fundus photography session. The eye exam included a check of visual acuity with pinhole refraction, if required. The anterior chamber of the eye was examined for contraindications to dilation or other abnormalities, and the intraocular pressure was measured. Dilating drops was administered (barring any contraindications), and a set of stereoscopic fundus photographs was taken for each eye. Patient completed brief questionnaire on history of eye disease and associated treatments at time of eye examination.

5.1.2 Visits 3-5 Annual followup questionnaire.
Questionnaire on eye disease or visual problems identified since the eye exam and photography in Visit 1 or 2 were administered by telephone or mail by WHI-SE staff on the first, second and third anniversary of the baseline eye examination through 3/30/05.

5.2 Eye Exam and Fundus Photography

Fundus photography was done using a 30 degree Zeiss FF series fundus camera. To avoid photographic artifacts, frequent inspection and cleaning of the front surface of the objective lens was essential to remove dust and debris. Professional Ektachrome Daylight film with a speed of 100 or slower was recommended by The Reading Center. It was also strongly recommended that Ektachrome films be processed by a certified “Q-Lab” to ensure consistent film processing quality. It was important that the processor correctly number the slide mounts to make slide sorting easier and more accurate.

5.2.1 Photographer Certification

All photographers taking photographs for this study were certified by the Reading Center for the seven standard fields protocol, before submitting actual patient photographs. Each clinical center was required to have at least one certified photographer, with preferably one or two additional photographers in order to participate.

5.2.2 Pupillary Dilation

Adequate dilation of the pupil was important to permit good quality stereo photography. Sufficient time was allowed for dilation to at least 6 mm, repeating drops if necessary, to achieve and maintain a pupil of at least this size during photography. Only if repeated instillation of drops and passage of at least 30 minutes after the last drops failed to produce dilation of 6 mm were photographs taken through a smaller pupil.

5.2.3 Required Color Photographs: The Standard Fields

Participants With Diabetes
The seven standard fields of the fundus, taken in stereo, of each eye were required for diabetic participants in the study. The following description assumes that there were two cross hairs in the camera ocular, one vertical and the other horizontal.

**Field 1** - Disc: Centered the optic disc at the intersection of the cross hairs in the ocular.

**Field 2** - Macula: Centered the macula at the intersection of the cross hairs in the ocular. In practice, to keep the central gray artifact created by some cameras from obscuring the center of the macula, the intersection of the cross hairs were placed about 1/8 - 1/4 DD nasal of the center of the macula.

**Field 3** - Temporal to Macula: Positioned the macula at the nasal edge of the field.

**Field 4** - Superior Temporal: The lower edge of the field was tangent to a horizontal line passing through upper edge of optic nerve and the nasal edge of the field was tangent to a vertical line passing through the center of the disc.

**Field 5** - Inferior Temporal: The upper edge of the field was tangent to a horizontal line passing through the lower edge of the optic disc and the nasal edge of the field was tangent to a vertical line passing through the center of the disc.

**Field 6** - Superior Nasal: The lower edge of the field was tangent to a horizontal line passing through the upper edge of optic disc and the temporal edge of the field was tangent to a vertical line passing through the center of the disc.

**Field 7** - Inferior Nasal: The upper edge of the field was tangent to a horizontal line passing through the lower edge of the optic disc and the temporal edge of the field was tangent to a vertical line passing through the center of the disc.

**Field 8** - An Optional Field: Outside the seven standard fields taken to document new vessels and/or preretinal or vitreous hemorrhage.

**Participants Without Diabetes**

A modified version of three standard fields of the fundus was required for the study, (two photos of the modified Field 1M and two photos of Field 2, both in stereo, and one modified photo of Field 3M were taken.) The modified three standard fields of the fundus specified by this protocol differ from the traditional Fields 1, 2, and 3, that were part of the seven standard field protocol, in the position of two fields. Field 1M and Field 3M were both modified to include the center of the macula; in Field 1M, near the edge of the field and in Field 3M, approximately midway between the edge and center of the field.

Field 1M was centered on the temporal edge of the optic disc, rather than on the center of the disc as in the traditional Field 1. Field 2 remained unchanged and was centered slightly above the center of the macula. Field 3M was centered about 3/4 to 1.0 disc diameter (DD) temporal to the center of the macula, so that the center of the macula was approximately midway between the center of the photograph and its nasal edge, rather than at its nasal edge (as in the traditional Field 3.) The modified three fields were illustrated in Figures 2a and 2b for the right and left eyes.
The following descriptions of the standard fields assume that there were two cross hairs in the camera ocular, one vertical and the other horizontal intersecting in the center of the ocular.

**Field 1M - Disc:** Centered the temporal edge of the optic disc at the intersection of the cross hairs in the ocular.

**Field 2 - Macula:** Centered the macula near the intersection of the cross hairs in the ocular. To keep the central gray artifact created by some cameras from obscuring the center of the macula, the intersection of the cross hairs were placed about 1/8 – 1/4 DD above the center of the macula. A suitable position could often be obtained by rotating the camera temporally from the Field 1M position, without vertical adjustment.

**Field 3M - Temporal to Macula:** Positioned the intersection of the cross hairs in the ocular 3/4 to 1.0 DD temporal to the center of the macula. If Field 2 was centered above the center of the macula, as suggested above, Field 3M may be centered 3/4 to 1.0 DD temporal to Field 2, a position easily achieved by rotating the camera without making any vertical adjustment or movement of the fixation device.

### 5.2.4 Fundus Reflex ("Lens") Photograph

A stereo fundus reflex photograph was taken in addition to those required of the seven standard fields (diabetic), and three modified standard fields (non-diabetic). As well as documenting the condition of the lens, the fundus reflex photograph allowed the Reading Center’s graders to take opacities of the media into consideration when reviewing photographic quality. If fundus photography was not possible because of opacities in the media, a bound-down pupil, previous enucleation, or for any other reason, a fundus reflex photograph was taken to document the reason.

In order to take the fundus reflex photograph, it was necessary to use the +16/+33 diopter setting on the auxiliary lens system of the Zeiss camera. The small white knob on the right side of the fundus camera was turned until the correct number (+20/+40 on some Zeiss cameras, perhaps similar high plus diopter readings on others) appeared at the dot. In order to standardize the magnification of these photographs (the object being to obtain a red reflex photograph magnified until the corneal diameter measures approximately 13 mm measured on the film), the following procedure was used:

1. The film-to-lens distance of the camera was increased to its maximum, by turning the large focusing knob so that its upper aspect moved toward the subject. The knob was turned in this direction as far as it would go. This adjustment determined the magnification.

2. The subject's headrest was moved away from the camera until the iris was in crisp focus (approximately one and a half inches further away than when adjusted for taking photographs of the fundus.) It was acceptable to move the subject slightly further away, so that the joystick could be used for fine adjustment of focus if the photographer wished. Focus was on lens opacities when present, otherwise on the pupillary margin.

3. The subject was asked to open his/her eyes very wide, or the lids were gently retracted if necessary, so that the entire cornea was visible.

4. The stereo photograph was taken.
After processing of film as slides, they were labeled and mounted into plastic slide sheets and sent to the UW Reading Center. The clinics sent fundus photographs to the University of Wisconsin labeled with the WHI ID number and a name code (the first three letters of the last name, followed by the first three letters of the first name).

5.2.5 Pathology Alert
All slides underwent a preliminary grading and detailed grading. During the preliminary grading, photographs were scanned for lesions which threaten vision or life and require immediate evaluation by the participant’s private physician or ophthalmologist.

Lesions that prompted a pathology alert were as follows—

1. Treatable age-related macular degeneration
2. Preproliferative/proliferative diabetic retinopathy
3. Clinically significant macular edema
4. Large cup to disc ratios suggestive of glaucoma
5. Large elevated nevus
6. Hollenhorst plaque (cholesterol emboli)
7. Macular hole
8. Retinal vascular occlusions
9. Miscellaneous lesions that may need evaluation for treatment

5.3 Grading Procedure at UW

The following describes the grading protocol used in the Women’s Health Initiative Sight Exam (WHI-SE). It was adapted from Beaver Dam Eye Study and represented a modification of the Wisconsin Age-related Maculopathy Grading System. It was divided into sections on the materials needed in grading, photographic processing procedures, identification information, evaluating photo quality, and grading of specific lesions for diabetic retinopathy, retinal arteriolar focal narrowing, arterio-venous nicking, age-related maculopathy, and other fundus lesions. The specific protocols used to take fundus photographs and to mail alerts regarding abnormal fundus pathology that required immediate feedback were described elsewhere. This section was limited to procedures involving photograph grading.

The grading involved a preliminary and detailed grading followed by a photograph edit and adjudication if necessary. Each set of photos was graded on the items detailed below using custom designed computer software with built in completeness and consistency checks. (An example paper grading form was included at the end of this protocol that mimics the direct entry grading system. Item numbers from the paper form were referenced throughout with right eye (OD) first followed by the left eye (OS) item number.) Upon completion of this detailed grading, a comparison was made between the preliminary grading and the detailed grading. If there was a disagreement in the levels assigned for specific lesions, the eye was sent to a third grader for an edited grade of those lesions. If the edited grade still did not agree with either the preliminary or detailed score, then the eye was sent to the consulting ophthalmologist for adjudication. The specific rules for edits/adjudications were under development.

The grader entered the ID# of the participant, the namecode, and photo date, the grader’s ID code and the date graded.
5.3.1 Preliminary Grading

5.3.1.1 Zeiss Photo quality

Overall Photo quality was graded as good (0), fair (1), borderline (2), poor-ungradable (3), or not applicable-no picture (9). Wisconsin Age-Related Maculopathy Grading System standards were used to characterize photo quality. This was an evaluation of overall photo quality that was assigned based on focus, field definition, stereopsis, photographic artifacts, and absence/presence of fields.

In grading photographic quality, a three-step scale was used. The steps, "good", "fair" and "poor", were defined below as they apply to a single photographic field.

<table>
<thead>
<tr>
<th>STEP</th>
<th>FIELD DEFINITION</th>
<th>FOCUS AND CLARITY</th>
<th>STEREO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>&lt; 2 DD from</td>
<td>Crisp (at least</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>definition</td>
<td>centrally)</td>
<td></td>
</tr>
<tr>
<td>Fair</td>
<td>2 to 1 DD from</td>
<td>Fuzzy, but better</td>
<td>Less than satis-</td>
</tr>
<tr>
<td></td>
<td>definition</td>
<td>than standard #14</td>
<td>factory but use-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ful for grading</td>
</tr>
<tr>
<td>Poor</td>
<td>More than 1 DD</td>
<td>Clarity no better</td>
<td>Little or no</td>
</tr>
<tr>
<td></td>
<td>from definition</td>
<td>than standard #14</td>
<td>stereo</td>
</tr>
</tbody>
</table>

A photographic field was considered "good" if all three characteristics listed above were graded "good"; "fair" if one of the conditions listed as "fair" was present and the other two were "fair" or "good"; and "poor" if one or more of the conditions listed as "poor" was present. Photo quality was also marked down when photographic artifacts were present in a field which affect the ability to evaluate retinopathy, or when the seven standard fields should have been provided but were incomplete.

A set of photographs was graded "good" (code "0"), "fair" (code "1"), "borderline" (code "2"), or "poor-ungradable" (code "3") according to the following criteria:

Good: Field 1 and 2 were of “good” quality, and four of the remaining five fields were of at least "fair" quality. (In patients where only three fields were taken Field 3 should be of “fair” quality.)

Fair: Field 2 was of “good” or “fair” quality, and Field 1 and four of the remaining five fields were of at least “fair” quality. (In patients where only three fields were taken, Field 3 could be any quality.)

Borderline: The photo set was judged acceptable for grading. Quality was poor, but proliferative lesions or significant non-proliferative lesions were evident. Large soft distinct drusen should also be gradable. Fields 1 and 2 should not be "poor".

Poor Ungradable: Quality was too poor to evaluate retinopathy and/or ARM status.

If the photographs were graded as fair, borderline, or poor-ungradable, then reasons for decreased quality were assessed. Focus, field, stereo, and other were considered. If something
was judged to be borderline/poor, but explainable due to severe lens opacities or other conditions, code 2 was chosen. If the assessment was borderline/poor but the reason for the poor quality was unexplainable, the grade was code 3. If any of these factors could not be assessed, the grade was code 8 (cannot grade [CG]). The “other” category was used for camera artifacts. If either of the borderline/poor codes were chosen, the grader details in the Comment section specifically what the artifact is, such as dust, haze, or arc.

Next, a decision was made whether retakes should be requested. This decision was based on whether the grader feels that there was no obvious reason that the photographs were borderline or poor-ungradable. Retakes were requested if it was thought that gradable photographs would result. If the WHI coordinators believe that retakes would not offer any better photographs, the OERC was notified and the original photographs submitted were graded according to protocol.

5.3.1.2 ARM Exclusions
While evaluating the photographs, the grader determined if there was a retinal condition that confounded the ability to grade the macula for age-related macular degeneration. If a confounding retinal condition existed the grader selected the most appropriate code for that condition. All of the ARM lesions were then coded “cannot grade” (code 8) and the grader continued grading the remaining items. In some cases a comment was included at the end of grading detailing the specifics of the exclusion condition.

Codes for ARM Exclusion

<table>
<thead>
<tr>
<th>Code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>1</td>
<td>Coloboma/Staphyloma</td>
</tr>
<tr>
<td>2</td>
<td>ROP</td>
</tr>
<tr>
<td>3</td>
<td>Vessel Occlusion</td>
</tr>
<tr>
<td>4</td>
<td>Dystrophy</td>
</tr>
<tr>
<td>5</td>
<td>Myopic Degeneration</td>
</tr>
<tr>
<td>6</td>
<td>Histo/Toxoplasmosis</td>
</tr>
<tr>
<td>7</td>
<td>Inflammatory Condition</td>
</tr>
<tr>
<td>8</td>
<td>Laser Rx in Macula</td>
</tr>
<tr>
<td>9</td>
<td>Non-ARM RPE Change</td>
</tr>
<tr>
<td>10</td>
<td>Non-ARM Detachment</td>
</tr>
<tr>
<td>11</td>
<td>Other</td>
</tr>
<tr>
<td>12</td>
<td>Unknown Etiology</td>
</tr>
<tr>
<td>13</td>
<td>Trauma</td>
</tr>
<tr>
<td>14</td>
<td>Vessel Occlusion</td>
</tr>
<tr>
<td>15</td>
<td>Maximum drusen size</td>
</tr>
<tr>
<td>16</td>
<td>Maximum drusen area</td>
</tr>
<tr>
<td>17</td>
<td>Maximum drusen type</td>
</tr>
</tbody>
</table>

5.3.1.3 Drusen
Drusen were described as round or ovate, sometimes slightly elevated deposits of variable size, were usually located in the plane of the retinal pigment epithelium (RPE). Three features of drusen, size, area of retinal involvement, and type were measured within the grid in the preliminary grading.

Maximum drusen size within the grid was measured as: none (0); questionable/stippling (1); <Std C₀ or 63μ in diameter (2); <Std C₁ or 125 μ in diameter (3); <Std C₂ or 250 μ in diameter (4); ≥Std C₂ and/or reticular drusen (5); and could not grade (8).

Drusen Area within the grid was categorized as:

- (0) None to minimal (no drusen or drusen < an area of a circle with a diameter of 105 μ)
- (1) Moderate drusen area (between 105 μ to < 500 μ diameter circle)
- (2) Substantial drusen area (≥ 500 μ diameter circle)
- (8) could not grade drusen area

Maximum drusen type was classified based on which drusen was considered to increase the risk of progression of progression to late ARM. Drusen type was measured as: none (0), hard
indistinct (1), hard distinct (2), soft distinct (3), soft indistinct/reticular drusen (4), and could not grade (8).

5.3.1.4 Other ARM Lesions

Other lesions (increased retinal pigment, retinal pigment epithelial depigmentation, geographic atrophy, PED/RD detachment, subretinal hemorrhage, subretinal fibrous or glial scarring, and evidence of local ablative photocoagulation treatment), which were described in more detail in the detailed grading section below, were graded as not being present (0), questionably present (1), present (2), and could not grade (8).

Other Lesions
This section was included to serve as an alert to the WHI coordinating centers regarding the need for further evaluation by an ophthalmologist or optometrist. After scanning the photographs, if there were no lesions which threaten vision or life (e.g., clinically significant macular edema, proliferative diabetic retinopathy, pre-proliferative diabetic retinopathy, recent branch or central retinal vein occlusion, cholesterol emboli (Hollenhorst plaque), macular hole or other rare lesions such as a retinal detachment or choroidal melanoma, the grade was no (0); if any such lesion(s) were found the appropriate grade, questionable (1), or present (2) was entered. Sometimes, photo quality or media changes did not permit evaluation of a lesion, then a could not grade (8) was entered.

Comments
Text was entered by the grader providing elaboration of other lesions and their location(s) that may need further evaluation or may be of interest.

5.3.2 Detailed Grading

Identification Information
The grader entered the ID# of the participant, the name code, and photo date, the grader’s ID code, and the date graded.

5.3.2.1 Zeiss Photo Quality
Fields 1, 2, 3 and 4-7 photographs were graded as absent (code 0) or present (code 2). If all were absent, all lesions were graded code 9. If present, the overall quality was assessed as borderline/poor (code 1) or as good/fair (code 2). The grader considers focus, field definition, stereo effect, and other artifacts. If any of these categories were assessed to be borderline/poor, code 1 was chosen. If the quality was poor but any question could be answered, the grade was coded 1. If any of these fields were completely ungradable, that is, 75% of the field was obscured, and no lesion was seen, the grade was coded 0.

Occasionally a participant had photographs taken with other types of cameras. If a different camera was used, protocols for grading were adapted from other suitable study protocols as necessary.
5.3.2.2 ARM Grading

5.3.2.2.1 ARM Exclude
If the eye was excluded from ARM grading due to a confounding condition, a notation was noted on the slide mount. If this was the case the detail grader coded this “Yes” (code 2), and all ARM items were coded “Cannot Grade” (code 8). If not, the grader chose “No” (code 0) and continued grading ARM lesions.

5.3.2.2.2 Drusen
Maximum Drusen Size
If there were no drusen visible within the grid in Field 2, the grade was none (code 0). If drusen were questionably present within the grid, (50% - 90% certain there were drusen present or there was an overall stippling texture to the retina), the grade was code 1. If there were drusen present, the grader determined the largest drusen present and compared this drusen with the Standard Circles (three sets of open circles). The grader chooses either: < Std. C₀ (63μ diameter), code 2; < Std. C₁ (125μ diameter), code 3; < Std. C₂ (250μ diameter), code 4; or ≥ Std. C₂ (250μ diameter), code 5; reticular drusen present, code 6. If the grader was unable to assess whether drusen were present within the grid in >75% of the field and no drusen were visible, the grade was CG code 8.

Number Of Subfields
The grader counted the number of subfields that contained the maximum size drusen (Code 2-6) and recorded a number from 1 to 9. If there were no or questionable drusen, or if drusen size could not be graded, no response was required as the item was automatically backfilled appropriately.

Drusen Area
If the grader found no drusen, the grade for drusen area was None/NA (code 0). If drusen were present within the grid in Field 2, the grader mentally moved together all the drusen present, regardless of size and configuration, and compared this area to the areas of Standard Circles. The grader chose either: < 63μ diameter, code 1, < 105μ diameter, code 2, < 250μ diameter, code 3, < 500μ diameter, code 4, < 1/2 disc area, 750μ diameter, code 5, < 1 disc area, 1500μ diameter, code 6, or equal to or greater to 1 disc area, code 7. If the grader was unable to judge the area of drusen present, the grade was CG, code 8.

Maximum Drusen Type
The grader chose the maximum drusen type present. If there were no drusen present the grade was none, code 0; if hard indistinct drusen were present the grade was code 1; if hard distinct drusen were present the grade was code 2; if soft distinct drusen were present the grade was code 3; if soft indistinct or reticular drusen were present the grade was code 4. If the type of drusen could not be assessed the grade was code 8.

Number Of Subfields Present With Maximum Type Drusen
The grader counted the number of subfields (0-9) with the maximum type drusen (hard distinct, soft distinct, or SI/Reticular only). If there were no or questionable drusen, or if drusen size/type could not be graded, no response was required as the item was automatically backfilled appropriately.
Drusen Grid Type
This item recorded all drusen types seen in each section of the grid; the central subfield, inner subfields, and outer subfields. The types of drusen considered were stippled or questionable, hard distinct, soft distinct, soft indistinct, and reticular. Each type of drusen was marked as absent, code 0; questionable, code 1; present, code 2; predominant by number (not area), code 3; and can’t grade, code 8.

5.3.2.2.3 Retinal Pigment Epithelial (RPE) Changes

Hyperpigmentation (Increased Retinal Pigment)
Hyperpigmentation was the clumping of granular grey or black pigment in or beneath the retina. If there was no hyperpigmentation, the grade was code 0. If questionably present, code 1 was chosen. If the total area of hyperpigment is: \(< \text{C}_0\), the grade was code 2; \(< \text{C}_1\), code 3; \(< \text{C}_2\), code 4; \(< \text{O}_2\), code 5; \(\geq \text{O}_2\), code 6. If there was hyperpigmentation not related to ARM present the grade was code 7. If the area within the grid could not be graded for hyperpigmentation, the grade was code 8.

Number of Subfields with Increased Pigment
The grader counted the number of subfields (0-9) with definite increased pigment present. If the code was none, questionable, pig/other, or could not grade, for Increased Pigment, then this was automatically backfilled.

Increased Pigment in Central Circle/Center Point (CC/CPT.)
If there was no increased pigment in the CC/CPT. of the grid in Field 2, the grade was code 0. If questionably present, code 1. If present in CC but not in the CPT, code 2. If present in CPT., code 3. If the presence of increased pigment could not be assessed for the CC/CPT., code 8.

RPE Depigmentation
The grader determined if there were any areas of RPE depigmentation. RPE depigmentation was usually characterized by a grayish-yellow or pinkish-yellow area of varying density and configuration with borders that gradually blend into areas of drusen or normal retina, often accompanied by hyperpigmentation. If there was no RPE depigmentation present, the grade was code 0. If questionably present, the grade was code 1. If definite RPE depigmentation, the grader compared the total depigmented area with the Standard Circles. If the total area was \(< \text{Std. C}_1\), the grade was code 2. If the area was less than \(\text{C}_2\), the grade was code 3. If the area was \(< \text{Std. O}_2\) the grade was code 4. If the area was less than 1/2 DA, the grade was code 5. If the area was less than 1 DA, the grade was code 6. If the area was equal to or greater than 1 DA, the grade was code 7. If RPE depigmentation could not be assessed in 75% of all fundus photographs present and none was present, the grade was CG.

Number of Subfields with RPE Depigmentation Present
The grader counted the number of subfields (0-9) with definite RPE depigmentation. If the code was none, questionable, or could not grade, for RPE Depigmentation, then this was automatically backfilled.

RPE Depigmentation-Central Circle/Center Point (CC/CPT.)
If there was no RPE depigmentation within the central circle area of the grid in Field 2, the grade was absent (code 0). If questionably present within the central circle, the grade was code 1. If definitely present within the central circle but not in the CPT, the grade was present (code 2).
If the central point was involved the grade was code 3. If the central circle area was not gradable and no RPE depigmentation was present, the grade was CG, code 8.

5.3.2.2.4 Other ARM Lesions

Late ARM Lesions
The grader answered the gatekeeper question, were there ARM late stage lesions present? If there were none, code 0 was chosen. If additional ARM was present or questionably present within any field, or if there were some lesions which should be marked CG, code 2 was chosen and the grader answers the next set of questions. If grading for other ARM lesions was not possible, CG code 8 was chosen.

Geographic Atrophy
Geographic atrophy was defined by the presence of one or more sharply-defined, usually more or less circular patches of partial or complete depigmentation of the RPE, which typically expose choroidal blood vessels. A patch must be greater than or equal to circle I, in total size to be considered. The grader considered whether there was geographic atrophy present in any field, in the center circle, and/or the center point. If none was present, the grade was code 0; if questionably present, code 1; if definitely present but not involving the center circle, code 2; if present in center circle but not in the center point, code 3; if present in center point, code 4; if CG, code 8.

Number of Disc Areas of Geographic Atrophy in Grid
If the grader indicated that geographic atrophy was definitely present, then the number of disc areas involved (1-16) were counted. Any definite amount less than 1 DA was counted as 1.

Pigment Epithelial Detachment (PED)/Detachment of Sensory Retina (RD)
Elevation of the retina was an important feature of ARM. Elevation of the retina occurs when it was pushed forward by accumulations of fluid, blood, or fibrovascular tissue between it and the RPE, or when the RPE and overlying sensory retina were pushed forward together by such accumulations (or mounds of drusen) between the RPE and the choroid. The grader considered whether any PED/RD detachments were present within all fields, in the center circle, and in the center point. If none were present, the grade was code 0; if questionably present, code 1; if definitely present but not involving the center circle, code 2; if present in center circle but not in the center point, code 3; if present in center point, code 4; if CG, code 8.

Subretinal Hemorrhage
Hemorrhage below the retinal surface may appear deep red or dark gray. The grader considered whether any subretinal hemorrhage was present within all fields, in the center circle and in the center point. If none were present, the grade was code 0; if questionably present, code 1; if definitely present but not involving the center circle, code 2; if present in center circle but not in the center point, code 3; if present in center point, code 4; if CG, code 8.

Subretinal Disciform Scar
Sheets or mounds of whitish material under the retina in eyes with ARM usually represent fibrous or fibrovascular tissue that has proliferated in areas previously occupied by serous or hemorrhagic subretinal fluid. The grader considered whether there was a disciform scar present in any field, in the center circle and in the center point. If none was present, the grade was code 0; if questionably present, code 1; if definitely present but not involving the center circle, code 2; if
present in center circle but not in the center point, code 3; if present in center point, code 4; if CG, code 8.

**ARM Treatment**
Laser treatment was sometimes used to treat choroidal new vessels associated with exudative ARM. Laser scars usually appear as localized areas of depigmentation often surrounded by scattered pigments. However, they were not always obvious. The grader considered whether any treatment scars for ARM were present within any fields, in the central circle, and in the center point. If none were present, the grade was code 0; if questionably present, code 1; if definitely present but not involving the center circle, code 2; if present in center circle but not in the center point, code 3; if present in center point, code 4; if CG, code 8.

**Number of Disc Areas of Exudative Lesions in Grid**
If the grader indicated that any exudative lesions were definitely present then the number of disc areas involved (1-16) was counted. Any definite amount less than 1 DA was counted as 1.

### 5.3.2.2.5 Retinal Vascular Lesions

**Focal Retinal Arteriolar Narrowing**
Focal areas of retinal arteriolar narrowing were easy to miss and require careful examination of all retinal arterioles present in all the fields, ignoring narrowing that was within 750μ (2 disc diameter) of the disc margin. If there was no retinal arteriolar narrowing seen, the grade was none (code 0). If the grader was between 50% to 90% certain that retinal arteriolar narrowing was present, the grade was questionable (code 1). If there was retinal arteriolar narrowing less than that seen in Standard Photograph #19 (<550μ), the grade was code 2. If there was retinal arteriolar narrowing Standard 19 (550μ =diameter of O2), the grade was code 3. If it was impossible to judge retinal arteriolar narrowing, the grade was CG. If there were multiple but separate areas of focal retinal arteriolar narrowing, the composite length of involvement was compared to the standard.

**Retinal Arteriole-Venule (A/V) Nicking**
Retinal A/V nicking, the constriction of caliber of a venule at an arteriole crossing, was noted. Tapering of the venous blood column on both sides of the crossing was required, ignoring nicking within 2 disc diameter (DD) of the disc margin. Codes are: absence of A/V nicking (code 0), questionable (code 1), present (code 2), and CG (code 8). If there were no areas where an arteriole crosses a venule, the grade was code 7. If no photos were present the grade was not applicable [NA] (code 9).

### 5.3.2.2.6 Other Retinal Lesions

**Other Lesions**
The grader answered the gatekeeper question, were other lesions present? If no other retinal or vitreal lesions were definitely or questionably present, the grade was no, code 0. If there were other retinal or vitreal lesions present, or questionably present (the grader was 50%-90% certain the lesion was, in fact, present), the grade was yes, code 2. If yes, the grader noted which specific lesion(s) from the list that follows was (are) questionably present, code 1; or definitely present, code 2; for all fields, and code 3 for some lesions in which the central point was involved.

**Other lesions include:**
Calcified Drusen
Peripheral Drusen
Peripapillary Atrophy
Arteriole Sheathing
Central Artery Occlusion
Branch Artery Occlusion
Central Vein Occlusion
Branch Vein Occlusion
Hollenhorst Plaque
Asteroid Hyalosis
Nevus
Chorioretinal Scar (CPT)
Surface Wrinkling Retinopathy-tension lines (CPT)
Surface Wrinkling Retinopathy-cellophane reflex
Macular Hole
Histoplasmosis (CPT)
Non-Diabetic Macular Edema (CPT)
Non-ARM Retinal Detachment (CPT)
Large C/D ratio
Thickened Vitreous/Vitreous Opacity
Other (note specific problem in Comments)(CPT)

5.3.2.2.7 Diabetic Retinopathy

5.3.2.2.7.1 Diabetic Retinopathy

When a participant has diabetes seven standard fields of stereo photographs were taken in order to adequately classify diabetic retinopathy severity. The Airlie House Classification system and the Early Treatment Diabetic Retinopathy Severity System were used to grade for the presence and severity of diabetic retinopathy. The severity scale was based on the presence and severity of a combination of lesions determined by comparison with standard photographs, and was an ordinal scale. Macular edema was not part of this scale.

Hemorrhages and Microaneurysms (HMA)

Microaneurysms, usually the first changes that define diabetic retinopathy that were seen on fundus photographs, appear as small circular red dots, varying in size from about 20 μ to 30 μ in diameter (they could never be larger than 125 μ in diameter). Retinal hemorrhages appear as spots of varying size with irregular margins and uneven densities. The grader chose the most severe score that was appropriate. The codes available to the grader were as follows:

0 None
1 Questionable
2 Definite MA’s only
3 Definite- HMA
4 \( \geq \) Std #1 (4 fields)
5 \( \geq \) Std 2A (1 field)
6 \( \geq \) Std 2A (2/3 fields)
7 \( \geq \) Std 2A (4 fields)
8 Cannot Grade
If seven standard fields were not available for grading, but the fields that were present were gradable, the options were limited to those answers that do not require field counts; that is, Definite, code 2 and \( \geq \) STD # 2A, code 3.

**Hard Exudate (HE)**

Retinal hard exudates were variable in size, sharply defined, and yellow. They may be scattered, aggregated, or “ring-like” in their distributions. If no HE was present, the grade was none, code 0. If HE was questionably present, the grade was code 1. If HE was present, the grade was code 2. If the presence of HE could not be assessed, the grade was code 8.

**Loops**

A venous loop was an abrupt, curving deviation of a vein from its normal path. If no venous loops were found the grade was code 0. If venous loops were questionably present, the grade was code 1. If a loop was present on a vein that was less than 31μ, the grade was code 2; if a loop was present on a vein that was greater than 31 μ the grade was code 3. If the presence of venous loops can’t be assessed the grade was code 8.

**Soft Exudate**

Soft exudates appear as whitish or grayish swelling in the nerve fiber layers of the retina due to small infarcts. If there was no soft exudate present, the grade was none, code 0; if questionably present, the grade was code 1; if definitely present, the grade was code 2; if could not grade lesion, code 8 was chosen.

**Intraretinal Microvascular Abnormality (IRMA)**

Intraretinal microvascular abnormality was a manifestation of retinal ischemia and appears as dilated capillaries (collateral vessels). If there was no IRMA present, the grade was 0; if questionably present, code 1. If IRMA was definitely present in less than 4 fields, the grade was code 2; if IRMA was present in 4 fields the grade was code 3; if IRMA was present equal to or greater than Standard 8A in any field the grade was code 4; if the presence of IRMA could not be assessed CG, code 8 was chosen.

If there were not seven standard fields present, the grader only choose those options that did not require field counts (Definite, code 2, \( \geq \) STD 2A).

**Venous Beading**

Venous beading was a sign of severe retinal ischemia. The retinal venules may vary from slight irregularity in caliber to “sausage-like” twisting. If there was no venous beading present, the grade was 0; if questionably present, code 1; if definitely present in only one field, code 2; if present in 2 or more fields, code 3; if CG, code 8 was chosen.

**5.3.2.7.2 Proliferative Diabetic Retinopathy**

Proliferative diabetic retinopathy was characterized by growth of abnormal blood vessels and fibrous tissue from the optic nerve head or from the inner retinal surface (usually on or near retinal venules.) The vessels, which appear initially as fine tufts on the surface of the retina subsequently grow into the outermost layer of the vitreous. Initially they consist of fine “naked” vessels which were permeable to fluorescein. They may hemorrhage into the vitreous.

**New Vessels on Disc (NVD)**
New vessels were considered to be “on the disc” if they were present within 2 DD of the disc margin. Once it was determined that new vessels were located within this region, the grader proceeds to measure the amount of neovascularization within 1 DD of the disc margin. If new vessels were present but there were no new vessels within 2 DD and only were outside of the 2 DD ring of the disc margin, they were to be graded as “new vessels elsewhere” (NVE). If there were no NVD, the grade was code 0. If NVD were questionably present, the grade was code 1. If there were NVD, but they were less than Standard 10A, the grade was code 2. If NVD equal or exceed Standard 10A, the grade was code 3. If NVD could not be assessed, the grade was code 8.

New Vessels Elsewhere (NVE)
If there were no NVE, the grade was code 0; if questionably present, the grade was code 1. If NVE were present and the area involved was less than 2 disc area, the grade was code 2. If NVE equals or exceeds 2 disc area, the grade was code 3. If NVE could not be assessed, the grade was code 8.

NVE Location
If NVE was graded as codes 1, 2 or 3, the location (Fields 1-7) where the NVE was present or questionably present was indicated. The options are; absent, code 0; questionable, code 1; present, code 2; or can=t grade, code 8.

Fibrous Proliferation (FP)
The mesenchymal cells responsible for the development of new blood vessels may also form fibrous tissue. Fibrous tissue predominates and may remain as the only evidence of proliferative retinopathy if regression of new vessels occurs later in the course of the disease. If there was no FP, the grade was code 0; if questionably present, the grade was code 1. If there was fibrous proliferation elsewhere (FPE), not on the disc or within 2 disc diameter (DD) of the disc, the grade was code 2. If there was fibrous proliferation on the disc (FPD) or within 2 DD of the disc, the grade was code 3. If there was both FPE and FPD, the grade was code 4. If FP could not be assessed, the grade was code 8.

Preretinal or Vitreous Hemorrhage (PRH or VH)
If there were no PRH or VH, the grade was none (code 0). If there was a questionable PRH or VH, the grade was code 1. If there was a PRH or VH less than 1 disc area (DA), the grade was code 2. If there was PRH or VH equal to or greater than 1 DA, the grade was code 3. If PRH and VH could not be assessed, the grade was code 8.
5.3.2.2.7.3 Diabetic Levels

Diabetic Retinal Level | Supporting Evidence
--- | ---
10 DR absent | 101 Microaneurysms and other lesions absent
12 Non-diabetic Retin. | 121 Diab. like lesions not related to diabetes.
13 Quest Retinop. | 131 Quest MA's and/or quest other lesions
14 HE, SE, IRMA, W/O MA's | 141 hard exudate, no microaneurysms
 | 142 Soft exudate, no microaneurysms
 | 143 IRMA, no microaneurysms
15 Hem only, no MA's | 151 Retinal hemorrhage, no microaneurysms
20 Microaneurysms only | 201 Microaneurysms only
31 Mild NPDR | 311 Venous loop ≥ code 1
 | 312 Questionable SE, IRMA or venous beading
 | 313 Retinal hemorrhage
37 Mild/Moderate NPDR | 371 Hard exudate
 | 372 Soft exudate
43 Moderate NPDR | 431 H/Ma ≥ Std Photo #1 in 4 or 5 fields
 | 432 H/Ma ≥ Std Photo 2A in 1 field
 | 433 IRMA in 1 to 5 fields
47 Moderately severe NPDR | 471 Both IRMA and H/Ma characteristics from level 43
 | 472 IRMA in 4 or 5 fields
 | 473 H/Ma ≥ Std Photo #2A in 2 or 3 fields
 | 474 Venous beading in one field
53 Severe NPDR | 531 Any two or three of level 47 characteristics
 | 532 H/Ma ≥ Std Photo #2A in 4 or 5 fields
 | 533 IRMA ≥ Std Photo #8A
 | 534 Venous beading in 2 or more fields
60 FP only | 601 FPD and/or FPE
61 No Ret and treatment | 611 same rules as level 10-15 AND scatter Rx present
62 MA’s only and treatment | 621 same rules as level 20 AND scatter Rx present
63 Mild NPDR and treatment | 631 same rules as level 31, or 37 AND scatter Rx present
64 Mod/Sev NPDR and treatment | 641 same rules as level 43, or 47 or 53 AND scatter Rx present
65 Mild/Moderate PDR | 651 NVE < 2 DA
 | 652 VH and/or PRH < 1 DA
Macular Edema (ME)
Increased permeability of retinal capillaries and retinal microaneurysms may result in an accumulation of extracellular fluid and thickening of the normally compact retinal tissue. Initially, there may be a slight loss of the normal transparency of the retina and the edema may be missed easily. The leakage and resulting edema may be focal around retinal microaneurysms or be diffuse and in some cases lead to the appearance of cystoid spaces in the outer retina. Clinically significant macular edema (CSME) was considered present when edema involved the fovea or was within 500 microns of the fovea, or when a 1+ disc area of edema was present with at least a portion of it within the macula.

If there was no ME present, the grade was code 0. If there was questionable ME, the grade was code 1. If ME was present but it was not clinically significant macular edema (CSME), the grade was code 2. If the ME present was CSME, the grade was code 3. If there was ME present but it was not related to diabetes, the grade was code 7. If ME could not be assessed, the grade was code 8.

Macular Edema-Central Circle (CC)
If there was no ME in the CC, the grade was code 0. If there was ME questionably present in the CC, the grade was code 1. If there was ME present in the CC without cysts, the grade was code 2. If ME was present in the CC with cysts, the grade was code 3. If there was ME in the CC but it was not related to diabetes, the grade was code 7. If ME in the CC could not be assessed, the grade was code 8.

Photocoagulation (PC) Scar
Local and/or scatter photocoagulation treatment was usually done to treat neovascularization (also retinal detachment) as a result of diabetes, retinal vein occlusion. If there were no photocoagulation scars the grade was none, code 0. If there were questionable OR incomplete scatter PC scars, the grade was code 1. If there were local PC scars, the grade was code 2. If scatter or panretinal photocoagulation (PRP) treatment was present, the grade was code 3. If scatter/PRP and local treatment were present the grade was code 4. If PC could not be assessed the grade was code 8.

Focal Photocoagulation (PC) Treatment
Focal laser photocoagulation, either as treatment of leaking microaneurysms (MA=s) or in a grid pattern, was done for the treatment of localized or diffuse or resistant macular edema. If there was no focal treatment the grade was none, code 0. If questionably present, the grade was code 1. If there was treatment for MAs only, the grade was code 2. If grid treatment only was present, code 3. If a combination of MA and grid treatment was present the grade was code 4. If focal treatment could not be assessed the grade was code 8.

5.3.3 Edits and Adjudication
5.3.3.1 Grading Edits
An edit was performed for macular drusen size, area, type, and the absence/presence of the following: increased retinal pigment, RPE depigmentation, geographic atrophy, PED/RD detachment, subretinal hemorrhage, subretinal scar, and laser Rx for ARM. Diabetic retinopathy levels based on the preliminary grading cut points was also compared for editing. If the preliminary grader and detail grader agreed on the lesions/levels that were compared the detailed grading data was copied to a table containing final grading data. When a disagreement was present in the grading of an eye, the lesions that disagree were regraded by a third grader masked to the previous grading data. The data collected from the edit grading was stored in an edit table. The edit grading was then compared to the preliminary and detail grading. If the edit grading agreed with either, the edit grading was copied to the final table of grading data. If no agreement was reached the eye underwent an adjudication by the consulting ophthalmologist.

5.3.3.2 Grading Adjudication
If an eye had lesions that needed adjudication, the consulting ophthalmologist was unmasked to all of the previous data collected for this study participant in order to make a final determination. The resulting data collected from the adjudication was stored in an adjudication table and also copied to the final table. The adjudicator may have chosen to change grades in any lesions or in the other eye of the same participant to resolve the disagreements and reach a satisfactory solution to a difficult eye/patient.

The fundus photograph results were entered into a database at the University of Wisconsin and periodically downloaded into the blinded database now at UCSF.

After close out of WHI main trial in 2005, HRT information was obtained and added to the WHI-SE data using the WHI ID number.
5.4 Efficacy Evaluation

ARM was evaluated using a modification of the Wisconsin Age-Related Maculopathy grading system. Severity of ARM in each eye was defined as follows:

- **Level 1**: No ARM (No signs of any ARM lesions or hard or soft drusen < 63 µm, without pigmentary abnormalities)

**Early ARM**

- **Level 2**: Minimal early ARM (Soft drusen ≥ 63 µm in diameter, with an area of drusen < 196,350 µm² and no pigmentary abnormalities or hard or soft drusen < 63 µm, with pigmentary abnormalities; no signs of late ARM)

- **Level 3**: Moderate early ARM (Soft drusen ≥ 125 µm in diameter, with an area of drusen ≥ 196,350 µm² and no pigmentary abnormalities or soft drusen ≥ 125 µm in diameter and an area of drusen < 196,350 µm², with pigmentary abnormalities present but no signs of late ARM)

- **Level 4**: Severe early ARM (Soft drusen ≥ 125 µm in diameter, with an area of drusen ≥ 196,350 µm² with pigmentary abnormalities present; no signs of late ARM)

**Late ARM**

- **Level 5**: Dry late ARM (Signs of geographic atrophy)

- **Level 6**: Wet late ARM (Signs of exudative macular degeneration)

A participant was defined as a ‘case’ of ARM if they had either a grading of 2 in both eyes or a grade of 3 or higher in one eye.

5.5 Laboratory determinations of biomarkers and genetic factors.

At enrollment into WHI-SE, participants were asked whether they consented to specimen collection for nutritional biomarker and genetic analyses. They may have consented to both types of analyses, limited their consent to just one type of analysis, or refused both. Blood samples for nutritional biomarkers were collected from consented WHI-SE participants once, around the time of their first eye examination. Clinics had the option of collecting fasting or non-fasting blood samples. For fasting samples, the participants fasted 12 hours before each test. Fasting or non-fasting status was indicated on the blood sample tracking form. A specimen for genetic analysis was collected as well, at the time of the biomarker collection. Approximately 32 ml of blood was drawn and stored for later use in substudies concerning biochemical, nutritional and genetic risk factors. All blood samples were labeled with sequential unique numbers provided to the clinic for blood samples. The blood samples for biomarker analysis included the
WHI ID# on the tube. The blood samples for genetic analysis were only labeled with the unique blood sample number. A separate data file linking the blood sample numbers and the participant ID is maintained by the Principal Investigator, Dr. Mary Haan. Laboratory testing facilities are unable to link the information on the tubes with any identifying information for the participant.

6 WITHDRAWAL FROM STUDY (DROPOUTS)
Patients withdrawn from the study were not replaced, regardless of the reason for withdrawal. An effort was made to determine why a patient failed to return for the necessary visits or was dropped from the study. This information was recorded on the patient's case report form.

7 SCREENING FAILURES
Patients who failed to meet the inclusion and exclusion criteria were defined as screen failures. A screening log, which documented the screening number, patient initials, and reason(s) for screen failure, is maintained by the investigator for all screen failures. A copy of the log was retained in the investigator’s study files.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical methods
The goal was to determine the effectiveness of HRT on ARM. Participants in the conjugated equine estrogens (CEE) group and the CEE combined with progestin (CEE+P) group were combined for evaluation of the treatment arm. ARM was not assessed prior to randomization, but WHI-SE participants were asked whether and when they were diagnosed as having ARM. This information was used to exclude participants who reported an ARM diagnosis before randomization from analyses. Logistic regression was used to test the association between randomization assignment and ARM after adjustment for relevant covariates. Possible explanatory variables included in the model were age, CEE vs CEE+P grouping, and relevant pre-existing co-morbid conditions, such as diabetes. The effect of clinical site was included if it was significant.

8.2 Sample Size and Power
Because WHI-SE was originally designed to include a second eye examination to capture ARM incidence, sample size calculations were based on the goal of comparing incidence of ARM in the HRT versus the control arm of the WHI HRT.

Sample size considerations are displayed in Table 2. Prevalence estimates of 5% for ARM were derived by reviewing results from available observational studies as described in Table 1. There are only two population-based studies that report the incidence of ARM. These have reported annual incidence of geographic atrophy or exudative disease at approximately 3-4% annually\(^ {25,26}\). If more inclusive measures are used, such as appear in Grade Level 3, the incidence may be higher. Only two published observational cohort studies examining the association between hormone replacement therapy (HRT) and ARM\(^ {3,12}\) These have reported a treatment
effect ranging from 30% to 40% reduction with use of HRT. There have been no randomized clinical trials examining the relationship between HRT and ARM. The outcome for this power analysis is a new diagnosis of late stage disease after the initial eye examination of any ARM. This approach does not exclude examining outcomes that require a smaller sample size such as changes in the number of drusen, size of the area of involvement, more inclusive classification system or progression from one stage to another. We projected a baseline sample size of 4400-4500 subjects, based on current recruitment projections by the local clinics. Table 2 shows the power available for several different combinations of treatment effects and sample sizes. Statistical power will be sufficient (80% or better) under most assumptions and slightly weak under the worse case assumption (n=4500, Treatment effect = 18%). The power to compare specific arms (estrogen vs. combined therapy) will be 80% or better if the treatment effect is at least a 25% reduction in risk of ARM.

| Table 2. Estimates of statistical power under several assumptions of treatment effects. |
|-------------------------------------------------|-----------------|-----------------|-----------------|
| Sample size range                               | 4500            | 5000            | 5500            |
| Prevalence of ARM 5%/20%                         | 225             | 250             | 275             |
| At risk (N)                                      | 4275            | 4750            | 5225            |
| Mortality years 1-4 5%/year                      | 793             | 881             | 969             |
| Alive at end                                     | 3482            | 3869            | 4256            |
| Number per treatment arm                         | 1741            | 1934            | 2128            |
| Incidence Per Year                               | Incident cases (N) Over 4 years |
| Placebo                                         | 4.80%           | 334             | 371             | 409             |
| Treatment effect:                                |                 |                 |                 |
| 18%                                             | 3.93%           | 274             | 305             | 335             |
| 20%                                             | 3.84%           | 267             | 297             | 327             |
| 25%                                             | 3.60%           | 251             | 279             | 306             |
| Power                                            |                 |                 |                 |
| 18%                                             | 76.8%           | 80.9%           | 84.4%           |
| 20%                                             | 85.1%           | 88.5%           | 91.2%           |
| 25%                                             | 96.7%           | 97.9%           | 98.7%           |

8.2.1 Analysis assumptions

8.2.1.1 Treatment Effects

The protective effect of HRT on the development of ARM was noted in two published studies. The Eye Disease Case-Control Study Group reported an odds ratio (OR) of 0.46 (95% confidence interval = 0.31 – 0.68) or a risk reduction of 54% and the Beaver Dam Eye Study reported an OR of 0.66 (0.26-1.67) in women aged 75+ or a risk reduction of 34%. Since these effect sizes were derived from observational studies and bias in these studies is likely to be high,
we calculated our sample sizes using the combined arms together with risk reductions ranging from 18% to 25%. Subjects taking ERT and PERT were combined and we assumed that there was no difference in effect by active treatment. As noted above, we were able to examine the sample by treatment arm as well if the treatment effect was 20% or better.

REFERENCES


