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## C-Reactive Protein and the Incidence of Macular Degeneration – Pooled Analysis of 5 Cohorts

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### Abstract

**Objectives**—To investigate the relationship between high-sensitivity C-reactive protein (hsCRP) and future risk of age-related macular degeneration (AMD) in US men and women.

**Methods**—We measured hsCRP in baseline blood samples from participants in five ongoing cohort studies. Patients were initially free of AMD. We prospectively identified 647 incident cases of AMD and selected age- and sex-matched controls for each AMD case (2 controls for each case with dry AMD, or 3 controls for each case of neovascular AMD). We used conditional logistic regression models to examine the relationship between hsCRP and AMD, and pooled findings using meta-analytic techniques.

**Results**—After adjusting for cigarette smoking status, participants with high (> 3 mg/L) compared with low (< 1 mg/L) hsCRP levels, had cohort-specific odds ratios (OR) for incident AMD ranging from 0.94 (95% CI 0.58-1.51) in the Physicians' Health Study to 2.59 (95% CI 0.58-11.67) in the Women's Antioxidant and Folic Acid Cardiovascular Study. After testing for heterogeneity between studies ( $Q=5.61$ ,  $p=0.23$ ), we pooled findings across cohorts, and observed a significantly increased risk of incident AMD for high versus low hsCRP levels (OR=1.49, 95% CI 1.06-2.08). Risk of neovascular AMD was also increased among those with high hsCRP levels (OR=1.84, 95% CI 1.14-2.98).

**Conclusion**—Overall these pooled findings from 5 prospective cohorts add further evidence that elevated levels of hsCRP predict greater future risk of AMD. This information might shed light on underlying mechanisms, and could be of clinical utility in the identification of persons at high risk of AMD who may benefit from increased adherence to lifestyle recommendations, eye examination schedules, and therapeutic protocols.

### INTRODUCTION

Inflammation plays a significant role in the incidence and progression of age-related macular degeneration (AMD),<sup>1,2,3</sup> the leading cause of blindness among older adults in the

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US.<sup>4</sup> Drusen, subretinal deposits indicative of the onset of AMD, have been shown to contain fibrinogen, vitronectin, complement components, and C-reactive protein (CRP), proteins associated with generalized inflammation.<sup>5,6,7</sup> Inflammatory cell debris has also been isolated from the outer surface of the Bruch's membrane in eyes with AMD.<sup>8</sup> The inflammatory hypothesis has been further strengthened by the discovery of a strong association between AMD and a common gene variant for complement factor H (CFH),<sup>9,10,11,12</sup> as well as variants in other complement pathway genes.<sup>13,14,15</sup>

C-reactive protein (CRP) activates the classic route of complement activation directly via cytokines through Fc receptor-binding by antibodies, which enhances the inflammatory response.<sup>16</sup> Circulating high-sensitivity C-reactive protein (hsCRP) levels have been widely studied as a nonspecific marker of systemic inflammation, and a single measure has been shown to reliably indicate the degree of underlying systemic inflammation in asymptomatic adults. Moreover, hsCRP blood levels have gained recognition through epidemiological studies as a useful clinical indicator of future cardiovascular risk.<sup>17,18</sup> Given the evidence linking inflammation and AMD, it has been of natural interest to determine whether hsCRP levels also are predictive of AMD. Prior studies of hsCRP and AMD<sup>19,20,21,22,23,24,25,26</sup> provide preliminary evidence of a relationship between hsCRP and AMD, but findings are mixed and there have only been three prospective studies. One of these prospective studies was of over 27,000 women in the Women's Health Study (WHS) cohort.<sup>27</sup> In this paper, we have extended these findings by conducting a pooled analysis of prospective nested case-control data from the WHS and four other cohorts.

## METHODS

### Study Population

The study population consisted of prospective nested case-control samples of participants in 5 population-based cohort studies (Women's Health Study [WHS], Physicians' Health Study [PHS], Women's Antioxidant and Folic Acid Cardiovascular Study [WAFACS], Nurse's Health Study [NHS], and Health Professional's Follow-up Study [HPFS]). The recruitment, enrollment, and characteristics of the study populations have been published elsewhere.<sup>28,29,30,31,32</sup> Briefly, the WHS, PHS, and WAFACS were all randomized, placebo-controlled trials initially designed to investigate the effect of aspirin or anti-oxidants on cardiovascular or cancer outcomes. The PHS consists of male physicians, while the WHS and WAFACS cohorts comprised female health professionals (primarily nurses). The HPFS and NHS were designed as observational cohort studies, consisting of male dentists, pharmacists and other health professionals in the HPFS and solely female nurses in the NHS. Participants in the PHS and WHS were apparently healthy men and women, respectively, who were free of prior diagnoses of cancer or cardiovascular disease. Women in the WAFACS cohort were at high risk of CVD, with a prior history of myocardial infarction or at least three major risk factors for CVD. Participants in the NHS and HPFS cohorts were not restricted from participating based on initial health status. Together, these cohorts include a total of 33 078 men and 67 093 women with stored baseline blood samples. A description of the cohorts is provided in Table 1.

At baseline, participants provided a baseline blood sample and completed a mailed questionnaire on which they reported demographic information as well as a medical history and personal information on a number of lifestyle factors, including height, weight, and cigarette-smoking history. Yearly follow-up questionnaires (every 2 years in the NHS and HPFS) provided reliable information on newly developed diseases and updated information on lifestyle factors. Subjects with prevalent AMD at baseline; subjects who did not provide a baseline blood specimen; or subjects for whom a hsCRP measurement was not available (due to various logistic reasons), were excluded from this analysis. The research protocol

was approved by the institutional review boards at Brigham & Women's Hospital and the Harvard School of Public Health.

### Confirmation of AMD

Procedures for our 2-stage documentation of incident AMD are nearly identical in each cohort, and have been previously described and validated.<sup>33,34,35</sup> On each study questionnaire, participants were asked to report any new diagnosis of AMD, including the month and year of diagnosis as well as the name and address of the diagnosing eye care professional, and for signed permission to review medical records. For each report of AMD, we sent a letter to the participant's ophthalmologist or optometrist to obtain information from the medical record on the date of diagnosis, best-corrected visual acuity at the time of diagnosis, date when visual acuity first reached 20/30 or worse in the affected eye, and the chorioretinal lesions that were present (drusen; retinal pigment epithelial [RPE] changes including atrophy, hypertrophy, and RPE detachment; geographic atrophy; subretinal neovascular membrane; or disciform scar). We confirmed a diagnosis of AMD for purposes of this study if one or more typical lesions were documented and associated with a visual acuity loss of 20/30 or worse. In those cases in which other ocular anomalies were also present, we asked the eye care professional to judge whether the visual acuity would be expected to be 20/30 or worse as a result of AMD alone. We defined neovascular AMD as the documented presence of an RPE detachment, sub-retinal neovascular membrane, or disciform scar that was not due to other causes (eg, histoplasmosis or choroidal rupture). Dry AMD included cases with the documented presence of drusen and/or retinal pigment epithelial changes but with no signs of neovascular AMD. We classified participants based on the most severely affected eye.

### Measurement of Biomarkers

Baseline blood specimens were collected and stored in liquid nitrogen freezers until the time of analysis, when samples were thawed and the levels of the inflammatory markers were measured in a core laboratory. Levels of hsCRP were analyzed using a validated immunoturbidimetric assay on the Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, Indiana) by using reagents and calibrators from Denka Seiken (Niigata, Japan).<sup>36</sup> A single technician, blinded to case-control status, performed all assays. Levels were similar to expected values for hsCRP in a population of healthy middle-aged men and women.<sup>37</sup>

### Case Control Selection

We employed prospective nested case-control designs within each of the 5 cohort studies in which cases of incident AMD were confirmed (as defined above). Controls were randomly selected from among those participants in the cohort who had not been diagnosed with AMD, and who had also provided a baseline blood specimen. Controls were matched to cases by age ( $\pm 1$  year), with up to 3 controls per case. We identified a total of 647 case-control sets who met our eligibility criteria.

### Statistical Analysis

We used conditional logistic regression to examine the relation of hsCRP levels with subsequent development of AMD. hsCRP levels were categorized using cutoff points of less than 1 mg/L, 1 to 3 mg/L, and greater than 3 mg/L, defined *a priori* based on the joint recommendation of the American Heart Association and the Centers for Disease Control and Prevention for clinical assessment of cardiovascular risk,<sup>38</sup> and for consistency with other recent studies of hsCRP and AMD, as well as for comparison with the literature on associations of hsCRP with cardiovascular disease.<sup>39</sup> In initial analyses, we obtained cohort-specific, smoking-adjusted odds ratios (ORs) and 95% confidence intervals (CIs) of AMD

for moderate (1-3 mg/L) and high levels (> 3 mg/L) of CRP compared to low levels (<1 mg/L) as a referent. All models included terms for the respective randomized treatment assignments depending on cohort (aspirin, beta-carotene, vitamin E, folic acid/vitamin B6/vitamin B12, vitamin C). We tested for linear trend across categories of the markers by entering a single ordinal score variable (0, 1, or 2) in the regression model. We then extended these models to adjust for other potential confounders including body mass index, use of antihypertensive and cholesterol lowering drugs, and dietary intake of omega-3 fatty acids, lutein/zeaxanthin, and zinc. Models were based on case-control sets for whom complete data were available on all covariates of interest. A two-tailed *p*-value of 0.05 was considered a statistically significant result. Pooled odds ratios were calculated with the random effects estimator and heterogeneity assessed with the Cochran Q test using STATA 10.1 (Stata Corp, College Station, TX, US). All other analyses were carried out using SAS 9.2 (SAS Institute Inc., Cary, NC, USA).

## RESULTS

Compared to control subjects, cases had significantly higher levels of hsCRP at baseline in each cohort except for the PHS, as well as higher body mass index (BMI) and a larger proportion of current cigarette smokers. The baseline characteristics of cases and controls from each cohort are shown in Table 2.

After adjusting for current smoking status, participants with high hsCRP levels had cohort-specific odds ratios of incident AMD ranging from 0.94 (95% CI 0.58-1.51) in the PHS to 2.59 (95% CI 0.58-11.67) in WAFACS; whereas participants with moderate hsCRP levels had cohort specific odds ratios of developing AMD ranging from 0.95 (95% CI 0.67-1.35) in the PHS to 1.54 (95% CI 0.80-2.94) in the HPFS, compared to participants with low hsCRP levels, Table 3. Further adjustment for other risk factors, including BMI and dietary intake of omega-3 fatty acids, lutein/zeaxanthin, and zinc, resulted in similar cohort-specific odds ratios for hsCRP and AMD, Table 3. No significant interactions (*P*-values > 0.15) were noted between hsCRP and randomized treatment assignment (aspirin, beta-carotene, vitamin E, vitamin C, or folic acid/vitamin B6/vitamin B12) in the case-control populations derived from randomized trials (PHS, WHS, and WAFACS). Adjustment for pack-years rather than current cigarette smoking did not impact the overall study findings (data not shown).

A formal test for heterogeneity between studies showed no statistically significant heterogeneity among the cohorts ( $Q = 5.61, p=0.23$ ). We therefore pooled results from all five cohort studies to obtain an overall estimate of the association between hsCRP and incident AMD (total  $N = 647$  incident cases of AMD and  $N = 1480$  controls). The combined odds ratios for AMD for increasing tertiles of hsCRP were 1.17 (95% CI, 0.92-1.47) and 1.48 (95% CI 1.06-2.08) compared to participants with low hsCRP levels, Figure 1.

Although the test for heterogeneity among cohorts was not significant, the observed findings from the PHS appeared qualitatively disparate compared with those of the other cohorts. We therefore probed the PHS data in an attempt to identify any factors that might help explain this observation. In particular, based on differences between the PHS and the other cohorts, we estimated the association of hsCRP with AMD in the PHS across: 1) quartiles of age at baseline, 2) randomized assignment to aspirin versus placebo, and 3) quartiles of the date of diagnosis of AMD during follow-up. These analyses failed to identify any important differences in the association between hsCRP and AMD by these variables. We also tested for heterogeneity by sex, calculating sex-specific odds ratios for AMD by pooling results from the three women-only cohorts, and separately for the two men-only cohorts. The odds ratios comparing individuals with high versus low levels of baseline hsCRP levels were 1.64

(95% CI 1.15-2.34) among women, and 1.39 (95% CI 0.59-3.29) in men, Table 4, however there was no evidence of heterogeneity in these findings ( $Q = 0.12$ ,  $p=0.72$ ).

Finally, we performed a separate analysis of the subset of cases with the neovascular form of AMD. There were an insufficient number of cases of neovascular AMD ( $N = 7$ ) in the WAFACS cohort to allow model convergence, so this cohort was not included in this analysis. In the 4 cohorts with sufficient numbers of cases, odds ratios comparing individuals with high versus low baseline hsCRP levels ranged from 1.64 (95% CI 0.75-3.58) in the NHS cohort to 2.32 (95% CI 0.76-7.10) in the HPFS, Table 5, with no evidence of heterogeneity ( $Q = 0.284$ ,  $p=0.963$ ). In pooled results from the 4 cohorts with sufficient numbers of cases of incident neovascular AMD ( $N= 183$  cases,  $N=546$  controls), the combined odds ratios for neovascular AMD were 1.84 (95% CI 1.14-2.98) for participants with high hsCRP levels, and 1.04 (95% CI 0.67-1.64) for participants with moderate hsCRP levels, compared to participants with low hsCRP levels, Figure 2.

## DISCUSSION

This analysis of five prospective case-control studies provides further evidence that a single measurement of hsCRP  $> 3$  mg/L predicts an increased risk of developing AMD over many years. After matching for age and controlling for cigarette smoking, individuals with baseline hsCRP levels  $> 3$  mg/L had a 50% increased risk of incident AMD, and a nearly 2-fold increased risk of neovascular AMD. In conjunction with other lines of evidence, these findings support the theory that low-grade systemic inflammation contributes to AMD development in the general population.<sup>40</sup>

The current study, pooling 647 incident cases of AMD and 1480 controls across five cohorts comprises the largest group of prospectively ascertained cases to date. Although the specificity of AMD diagnosis is high in these cohorts,<sup>41</sup> ascertainment of AMD cases may have been incomplete. However, the impact of any missed cases would be minimal in this prospective nested case-control study. For most cases included in the present study, the type or size of drusen was not collected when confirming cases, which might lead to some misclassification of AMD, though other analyses based on these same cases and controls have shown strong and consistent associations with known genetic and non-genetic AMD risk factors suggesting any such bias is likely to be small. Inclusion of AMD cases and controls from large prospective cohorts of US men and women improves the generalizability of conclusions, but the inclusion of only health professionals may limit the generalizability, particularly if the distribution of hsCRP levels is shifted toward lower levels as might be expected if these populations of health professionals are more healthy than the general population.

There was some variability in estimates of association among the five cohorts, but this heterogeneity was not statistically significant, and was not apparent in subgroup analyses of the more severe neovascular AMD cases. Investigation of possible sources of heterogeneity identified a stronger association in the 3 cohorts with more recent blood collections as compared to the 2 cohorts (PHS and NHS) with blood collections in the 1980s. Although the stability of hsCRP in frozen blood samples has been previously demonstrated,<sup>42</sup> there is always a possibility that some degradation of hsCRP occurred in the stored samples. The design of the study precludes any direct analysis of this potential source of bias, but the most likely impact of degradation over time would be a null-ward bias in associations.

Previous cross-sectional studies of the relationship between hsCRP and AMD have provided mixed results. Two clinic-based studies<sup>19,43</sup> noted an association between hsCRP level and AMD, while one cross-sectional population-based study did not.<sup>44</sup> The increased likelihood

of selection bias among cross-sectional and clinic-based studies lends greater weight to the conclusions of more recent prospective studies, which minimize selection and surveillance bias, and also provide information on hsCRP levels prior to the onset of AMD. Of three recent population-based prospective studies, one noted no association;<sup>45</sup> whereas two others<sup>46,47</sup> reported an increased incidence of AMD among individuals with higher baseline levels of hsCRP. One of these studies (Schaumberg et al.) was a full cohort analysis of WHS, and therefore included the same set of incident AMD cases that are included here in the nested case-control sample from that population. That study found a 90% increased incidence of AMD among women with hsCRP > 3 mg/L. The results from the WHS case-control analysis in this study were consistent with the full WHS cohort analysis. In the other prospective study in which a significant association was observed, Boekhoorn et al. noted a 40% increased incidence of early AMD among persons with hsCRP > 3.26 mg/L and an 80% increased incidence of late AMD cases (neovascular AMD and central geographic atrophy) among persons with hsCRP > 3.23 mg/L each compared to persons with hsCRP < 0.83 mg/L. In a study of 254 individuals with early AMD at baseline, Robman et al. recently observed an 80% increased risk of AMD progression over 7 years.<sup>48</sup> Our results are also consistent with a recent meta-analysis (Hong et al.) of 11 studies (9 cross-sectional and 2 prospective), in which the a two-fold increase in late AMD (primarily neovascular cases) and a 31% increase in overall AMD in subjects with elevated hsCRP > 3mg/L.<sup>49</sup>

CRP has emerged over the past decade as an important risk marker for cardiovascular and other age-related diseases. Data from the WHS, one of the five cohorts included here, for example, showed a 66% increased risk of coronary heart disease among women with baseline hsCRP > 3 mg/L;<sup>50</sup> a magnitude similar to the increased risk of AMD we previously observed in the WHS, as well as in pooled findings from the five cohorts in this report. Although a role of inflammation and innate immunity/complement dysregulation in AMD is now established,<sup>51,52,53,54</sup> a direct role for CRP in AMD causation remains a topic of research and debate.

The ability of CRP to induce complement activation, coupled with the presence of complement components in subretinal drusen suggests a possible etiological role of CRP in the pathogenesis of AMD. Supporting this hypothesis, prior laboratory work has shown that the common AMD-associated Y402H variant of complement factor H (CFH) attenuates its binding affinity for CRP, particularly at higher concentrations of CRP,<sup>55</sup> and thus reduces deactivation of the complement cascade. This may result, at least in theory, in alterations in the RPE, damage to the underlying Bruch membrane, and deposition of drusen and progression to AMD.<sup>56,57,58</sup> Such findings concur with evidence from a recent epidemiological study, which suggests that risks of AMD associated with higher hsCRP levels are greater among individuals with the Y402H genotype.<sup>59</sup> We think it is important to continue to study whether CRP interacts with CFH or other AMD-associated genes, and plays a direct role in any of these pathways, since pharmacologic modification of CRP levels is a possibility. However, the use of statin drugs to lower CRP levels, as is being tested for prevention of cardiovascular outcomes, does not appear promising for AMD in light of good evidence that such drugs have no effect on AMD progression.<sup>60</sup>

In conclusion, our pooled data from 5 prospective studies demonstrate that persons with an elevated hsCRP level > 3 mg/L (a level used to indicate increased risk of cardiovascular disease) have a higher incidence of AMD. Given these findings, and the similar results of a recent meta-analysis,<sup>52</sup> studies might be considered, for example, to determine whether measurement of hsCRP could be useful to motivate individuals with higher risk levels to make lifestyle changes (e.g., smoking cessation, dietary modification, weight loss) or present for regular eye exams so that any interventions to prevent vision loss from AMD could be initiated in a timely fashion. These data also support continued investigation of the

possibility that hsCRP may contribute in some way to the pathogenesis of AMD. If shown, such a finding would further support interventions to lower systemic CRP levels to prevent AMD onset and progression.

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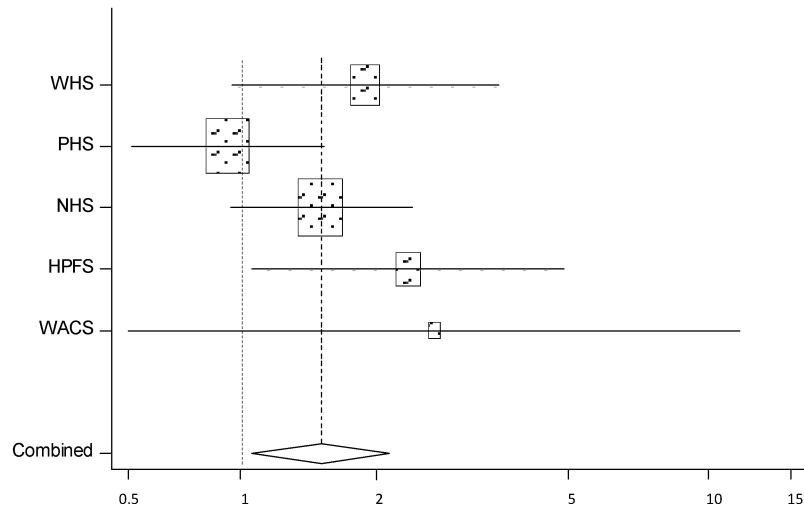
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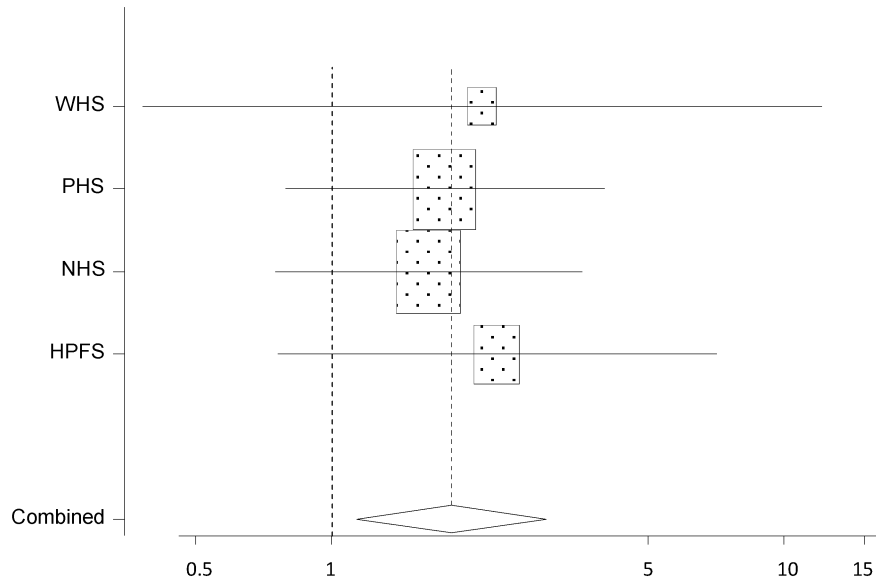
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**Figure 1.** Smoking Adjusted Odds Ratios (vertical midline of each box) and 95% confidence intervals (horizontal bars) for the association between hsCRP levels  $>3$  mg/L (versus  $<1$  mg/L) and risk of AMD in nested case-control samples from five prospective cohorts. The size of the squares is proportional to the inverse of the variance of the ORs (and reflects sample size). The diamond represents the summary odds ratio estimate (center of diamond) and 95% confidence interval for the pooled estimate (horizontal points of diamond).



**Figure 2.** Smoking Adjusted Odds Ratios (vertical midline of each box) and 95% confidence intervals (horizontal bars) for the association between hsCRP levels >3 mg/L (versus <1 mg/L) and risk of neovascular AMD in nested case-control samples from 4 prospective cohorts. The size of the squares is proportional to the inverse of the variance of the ORs (and reflects sample size). The diamond represents the summary odds ratio estimate (center of diamond) and 95% confidence interval for the pooled estimate (horizontal points of diamond).

**Table 1**  
**Details of Prospective Cohorts included in the Present Analyses**

| Study  | Study Description  | Year of Inception | Participants   | Number with blood samples | Years of Blood Collection | Follow-up Years |
|--|--|-------------------|--|---------------------------|---------------------------|-----------------|
| Physicians' Health Study (PHS)                                   | Randomized clinical trial of aspirin and beta-carotene   | 1982              | 22,071 male physicians, aged 40-84 in 1982, free of prior CVD or cancer                              | 15,124                    | 1982-1984                 | 20.8            |
| Nurses' Health Study (NHS)                                       | Prospective cohort study                                 | 1976              | 121,700 female registered nurses, aged 30-55 in 1976   | 32,826                    | 1989-1990                 | 16.4            |
| Health Professionals Follow-up Study (HPFS)                      | Prospective cohort study                                 | 1986              | 51,529 male health professionals, aged 40-75 in 1986   | 18,018                    | 1993-1994                 | 10.5            |
| Women's Health Study (WHS)                                       | Randomized clinical trial of aspirin and vitamin E       | 1992              | 39,876 female health professionals, aged 40 or older in 1992, free of prior CVD or cancer            | 28,345                    | 1993-1996                 | 10.6            |
| Women's Antioxidant and Folic Acid Cardiovascular Study (WAFACS) | Randomized clinical trial of antioxidants and B-vitamins | 1996              | 8,171 female health professionals, aged 40 or older with CVD or at least three coronary risk factors | 5,922                     | 1993-1996                 | 9               |

**Table 2**  
**Baseline Characteristics of the Nested Case-Control Study Populations**

|                                   | Male Cohorts |          | Female Cohorts |          |       |          | WHS   | WAFACS   |      |
|-----------------------------------|--------------|----------|----------------|----------|-------|----------|-------|----------|------|
|                                   | PHS          |          | HPFS           |          | NHS   |          |       |          |      |
|                                   | Cases        | Controls | Cases          | Controls | Cases | Controls | Cases | Controls |      |
| Age at Baseline blood collection* | Mean         | 63.3     | 62.9           | 64.8     | 64.9  | 61.5     | 66.1  | 69.3     | 69.2 |
|                                   | Std Dev      | 7.3      | 7.0            | 5.0      | 5.0   | 4.5      | 6.6   | 5.3      | 5.2  |
| Body Mass Index.                  | Mean         | 24.6     | 24.6           | 25.3     | 25.0  | 25.4     | 26.2  | 28.6     | 27.9 |
|                                   | Std Dev      | 2.8      | 2.6            | 2.8      | 2.8   | 4.9      | 5.0   | 5.3      | 5.5  |
| Current Cigarette Smoking         | %            | 10.5%    | 8.5%           | 7.7%     | 6.9%  | 25.0%    | 14.2% | 20.0%    | 6.5% |
| hsCRP (mg/L)                      | Mean         | 1.9      | 2.1            | 2.4      | 1.7   | 3.8      | 5.4   | 8.8      | 4.2  |
|                                   | Std Dev      | 3.9      | 3.8            | 2.4      | 1.6   | 5.2      | 8.1   | 10.1     | 4.4  |

\* Cases and controls were matched on age during contro selection

**Table 3**  
**Odds Ratios and 95% Confidence Intervals for AMD among initially healthy participants in five prospective studies according to hsCRP level**

|                      | Odds Ratio (95% Confidence Interval) |                  |                  |                  |                   |
|----------------------|--------------------------------------|------------------|------------------|------------------|-------------------|
|                      | PHS                                  | HPFS             | NHS              | WHS              | WAFACS            |
| hsCRP (mg/L)         |                                      |                  |                  |                  |                   |
| Model 1 *            |                                      |                  |                  |                  |                   |
| < 1                  | 1 [Reference]                        | 1 [Reference]    | 1 [Reference]    | 1 [Reference]    | 1 [Reference]     |
| 1 – 3                | 0.95 (0.67-1.35)                     | 1.54 (0.80-2.94) | 1.28 (0.82-1.98) | 1.47 (0.77-2.82) | 1.18 (0.21-6.79)  |
| > 3                  | 0.94 (0.58-1.51)                     | 2.28 (1.06-4.91) | 1.49 (0.95-2.31) | 1.85 (0.96-3.56) | 2.59 (0.58-11.67) |
| p-trend <sup>‡</sup> | 0.75                                 | 0.03             | 0.08             | 0.07             | 0.13              |
| Model 2 †            |                                      |                  |                  |                  |                   |
| < 1                  | 1 [Reference]                        | 1 [Reference]    | 1 [Reference]    | 1 [Reference]    | 1 [Reference]     |
| 1 – 3                | 0.99 (0.67-1.46)                     | 1.34 (0.64-2.77) | 1.30 (0.82-2.07) | 1.44 (0.72-2.90) | 1.37 (0.17-10.87) |
| > 3                  | 0.89 (0.52-1.53)                     | 2.27 (0.95-5.40) | 1.32 (0.81-2.14) | 1.75 (0.84-3.68) | 1.98 (0.30-13.16) |
| p-trend <sup>‡</sup> | 0.71                                 | 0.07             | 0.27             | 0.14             | 0.44              |

Abbreviations: WHS, Women's Health Study; PHS, Physician's Health Study; NHS, Nurse's Health Study; HPFS, Health Professional's Follow-Up Study; WAFACS, Women's Antioxidant and Folic Acid Cardiovascular Study. hsCRP, high-sensitivity C-reactive protein

\* Model 1: Adjusted for randomized treatment assignment and current cigarette smoking. 9 cases and 18 controls from the WAFACS study were dropped from model 1 because of lack of folate randomization status

† Model 2: Adjusted for factors in model 1 and additionally for antihypertensive and cholesterol lowering drugs; body mass index (calculated as weight in kilograms divided by height in meters squared); and dietary intake of omega-3 fatty acids, lutein/zeaxanthin, and zinc. The analysis in model 2 was limited to subjects with data on covariates of interest (i.e. dietary data, medications, etc). 45 cases and 102 controls were dropped from model 2 because of lack of covariate data

‡ P Value represents result of testing for a linear trend by the inclusion of an ordinal score variable (0, 1 or 2) in regression models



**Table 4**  
**Smoking Adjusted Odds Ratios and 95% CIs for AMD – By Gender**

|            | Men <sup>*</sup> | Women <sup>+</sup> | Heterogeneity      |
|------------|------------------|--------------------|--------------------|
| CRP (mg/L) |                  |                    |                    |
| < 1        | 1 [Reference]    | 1 [Reference]      |                    |
| 1 - 3      | 1.12 (0.71-1.74) | 1.33 (0.93-1.90)   | Q = 0.37, p = 0.55 |
| > 3        | 1.39 (0.59-3.29) | 1.63 (1.15-2.34)   | Q = 0.12, p = 0.73 |

\*The PHS and HPFS were contributors to the male grouping

<sup>+</sup>The WHS, NHS, and WAFACS were contributors to the female grouping

**Table 5**  
**Smoking Adjusted Odds Ratios and 95% CIs for Neovascular/Wet AMD<sup>†</sup>**

|            | PHS              | HPFS             | NHS              | WHS               |
|------------|------------------|------------------|------------------|-------------------|
| CRP (mg/L) | N = 69 cases     | N = 28 cases     | N = 61 cases     | N = 25 cases      |
| < 1        | 1 [Reference]    | 1 [Reference]    | 1 [Reference]    | 1 [Reference]     |
| 1 - 3      | 0.87 (0.46-1.64) | 0.98 (0.31-3.06) | 1.13 (0.48-2.68) | 3.33 (0.59-18.67) |
| > 3        | 1.78 (0.79-4.00) | 2.32 (0.76-7.10) | 1.64 (0.75-3.58) | 2.15 (0.36-12.68) |
| p-trend    | 0.32             | 0.13             | 0.19             | 0.81              |

<sup>†</sup>There was an insufficient number of cases of wet AMD in the WAFACS cohort (N = 7), so this cohort was excluded from this analysis.