AMERICAN ACADEMY OF OPHTHALMOLOGY®

Oxidized Low-density Lipoprotein and the Incidence of Age-related Macular Degeneration

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Purpose: To examine the relationship between serum oxidized low-density lipoprotein (ox-LDL) cholesterol and the incidence of age-related macular degeneration (AMD) over a 25-year period in a sample of persons from the population-based Beaver Dam Eye Study (BDES).

Design: Observational prospective cohort study.

Participants: A total of 4972 people from the BDES (aged 43–84 years and living in Beaver Dam, Wisconsin in 1988) seen during at least 1 of 6 examination phases at approximately 5-year intervals between 1988 and 2016.

Methods: A 50% random sample of participants (N = 2468) was selected for ox-LDL measurements. Stored frozen specimens from every examination phase were processed using an enzyme-linked immunosorbent assay from a single batch. All available intervals were included for a person, resulting in 6586 person-visits.

Main Outcome Measures: Age-related macular degeneration was assessed using the Wisconsin Age-related Maculopathy Grading System, and severity was defined using a 5-step severity scale. The severity of the worse eye at each examination was used for analyses. A multi-state Markov (MSM) model was fit to simultaneously assess the ox-LDL relationship to all AMD transitions, including incidence of any AMD, incidence of late AMD, and worsening and improvement of AMD over the 25 years of the study.

Results: The mean (standard deviation) level of ox-LDL was 75.3 (23.1) U/L at the baseline examination. When adjusting for age, sex, ARMS2 and CFH risk alleles, and examination phase, the ox-LDL at the beginning of a period was not statistically significantly associated with the incidence of any AMD (hazard ratio per 10 U/L ox-LDL was 1.03, 95% confidence interval 0.98,1.09). Furthermore, ox-LDL was not associated with worsening anywhere along the AMD severity scale, nor with incidence of late AMD. The lack of relationships of ox-LDL to the incidence of any AMD or worsening of AMD remained after adjustment for history of statin use, smoking status, body mass index, and history of cardiovascular disease (data not shown).

Conclusions: Our findings do not provide evidence for statistically significant relationships between ox-LDL and AMD disease development or worsening of AMD. *Ophthalmology 2019;126:752-758 © 2018 by the American Academy of Ophthalmology*

Despite new medical and surgical interventions, age-related macular degeneration (AMD) remains a leading cause of vision loss in people 65 years of age or older in the United States.[1,2] Although there is a growing body of information regarding the natural history of AMD and its relation to risk factors, gaps still remain in our understanding of its earliest stages and what factors are associated with risk for its incidence and progression.[3–5] This is important, as the treatment for late neovascular AMD carries some risks, may not be permanent, and is costly.[6] In addition, there are no treatment options for geographic atrophy.[7] Thus, there is need for additional therapeutic approaches at early stages of the disease to delay or prevent worsening to late stages of AMD, both neovascular and geographic atrophy.[3]

Because the oxidative process is thought to be linked to AMD, it is hypothesized that oxidized serum low-density lipoprotein (ox-LDL) would be a reasonable biomarker of oxidative stress in the retina, rather than native serum LDL.[8–19] To our knowledge there are no population-based long-term cohort studies that have examined the association of serum ox-LDL cholesterol with the incidence and worsening of AMD. To this end, our objective was to determine the relationships of a modifiable risk factor, ox-LDL cholesterol, to the incidence and worsening of AMD[20] in the Beaver Dam Eye Study (BDES).

Methods

Study Population

Detailed descriptions of the BDES cohort, participation statistics, and reasons for nonparticipation have appeared elsewhere.[21–26] From 1987 to 1988, a private census of the city and township of Beaver Dam, Wisconsin identified all 5924 individuals in the target

https://doi.org/10.1016/j.ophtha.2018.12.026
ISSN 0161-6420/19

age range (43−84 years), and these persons were invited to have a baseline examination (BDES1) and up to 5 follow-up examinations (BDES2−6) at approximately 5-year intervals. Approximately 80% of surviving eligible individuals participated during each examination phase. There were 4972 people seen at any examination phase (4926 seen at baseline, 3721 at BDES2, 2962 at BDES3, 2375 at BDES4, 1913 at BDES5, and 1181 at BDES6). A 50% random sample of these 4972 people was selected, regardless of the availability of samples or number of examinations. All visits for the 2468 selected individuals were eligible for analyses (2440 seen at baseline, 1867 at BDES2, 1464 at BDES3, 1196 at BDES4, 948 at BDES5, and 604 at BDES6). The information across all examination phases was used, resulting in 8519 of the 17 078 person-visits (50%). Examinations were done at the clinic as well as off-site with participating clinics, nursing homes, and some in-home examinations for homebound individuals. The same protocols were used at all locations. Ninety-nine percent of the cohort was white.

Approval was granted by the institutional review board at the University of Wisconsin. Written informed consent was also granted by the institutional review board for the use and disclosure of protected health information, which was obtained from all subjects before being enrolled in the study and before each examination. The study was performed in accordance with the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

Examination

Pertinent parts of the examination included the measurements of blood pressure, height, and weight and the recording of answers to questions regarding the use of tobacco products and the consumption of alcoholic drinks. A history of using lipid-lowering medications was also ascertained.

Laboratory Procedures for Oxidized Serum Low-density Lipoprotein

Nonfasting blood samples were collected at all BDES examinations, with the exception of BDES4. The blood samples were processed within 1 hour of collection, and serum was frozen at −80° C. A participant could have stored samples from up to 5 visits. After the BDES6 examination phase, 6431 frozen serum samples from the randomly selected participants were sent to the University of Minnesota Advanced Research and Diagnostic Laboratory (Minneapolis MN). Oxidized serum LDL was measured in serum using a solid phase 2-site enzyme-linked immunosorbent assay (ox-LDL ELISA enzyme immunoassay kit, Lot #25806, Mercodia AB, Uppsala, Sweden). Serum samples were automatically pipetted using a Beckman Coulter Biomek NXp instrument (Beckman Coulter, Fullerton, CA). The intensity of the color was measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, CA). The interassay laboratory coefficient of variation for the ox-LDL method for an in-house pooled serum control was 6.9% at a mean concentration of 48.9 U/L. Serum samples have shown excellent long-term stability when continuously stored at −80° C.

Assessment of Presence and Severity of Age-related Macular Degeneration

After the participants' pupils were dilated, 30° stereoscopic color film photographs of the Early Treatment Diabetic Retinopathy Study 3 standard fields of the retina (field 1 centered on the optic disc, field 2 centered on the fovea, and nonstereo field 3 centered temporal to field 2) were obtained for each eye at each examination. The Wisconsin Age-related Maculopathy classification system was used for grading of AMD lesions, such as on drusen size, type and area of involvement, signs of pigmentary abnormalities (increased retinal pigment and retinal pigment epithelium [RPE] depigmentation), and signs of geographic atrophy and/or signs of exudative AMD. Eyes with dramatic changes (disappearance of AMD lesions) between visits were reviewed, masked to visit order, to confirm the changes were real. Disappearance of drusen does happen, but most other disappearance occurs when lesions are not visible within the image anymore (e.g., detachments that have settled, etc.). The 3-Continent 5-step AMD severity scale was used to define the presence and severity of AMD.[27] Eyes were defined as having no AMD (level 10), minimally severe early AMD (level 20), moderately severe early AMD (level 30), severe early AMD (level 40), and late AMD (level 50). Analyses were done based on the severity in the worse eye.

Definitions

Age was defined as age at the time of each examination. Systolic and diastolic blood pressures were defined as the average of the 2 measurements taken according to the Hypertension Detection and Follow-up Program protocol.[28] Hypertension was defined as blood pressure of 140/90 mmHg or current use of blood pressure−lowering medication. A current smoker was defined as a person who had smoked more than 100 cigarettes in his or her lifetime and reports still smoking. Heavy drinking was defined as ever having a period of time where the subject consumed 4 or more drinks a day, on average. Body mass index (BMI) was defined as the participant's weight in kilograms divided by height in meters squared. History of cardiovascular disease was defined based on self-reported history of angina, myocardial infarction, or stroke. Glycosylated hemoglobin was measured at each examination and diabetes was defined based on self-report and/or a glycosylated hemoglobin A1c >6.5%. Serum creatinine was measured by an enzymatic method (CREA plus; Roche Diagnostics, Indianapolis, IN) using the Roche Modular P Chemistry Analyzer (Roche Diagnostics), consistent with the current National Kidney Disease Education Program recommendations for standardizing serum creatinine measurement.[29] Estimated glomerular filtration rate (eGFR) was calculated using the chronic kidney disease epidemiology (CKD-EPI) formula.[30] Serum total cholesterol and high-density lipoprotein (HDL) cholesterol were measured at BDES1 and BDES2 and were obtained through an ancillary study (Epidemiology of Hearing Loss Study)[31] conducted at the same time as the BDES3 examination phase. Measurement of native LDL cholesterol was not available for this population. The A69S polymorphism of the *ARMS2* gene (rs 10490924) and the Y402H polymorphism of the *CHF* gene (rs 1061170) were available in the population. The number of risk alleles from each polymorphism was added and the risk was coded as low (0−1 risk alleles), moderate (2 risk alleles), and high (3−4 risk alleles).[32]

Statistics

Comparisons of baseline characteristics for persons selected for the ox-LDL measurements were done using standard regression (linear regression for continuous measures and logistic regression for binary measures). Multi-state Markov (MSM) models were used to evaluate the associations of known and potential risk factors with the incidence, worsening, and improving of AMD in continuous time. At any given age, subjects belonged to 1 of 6 possible states: death or 1 of the 5 AMD severity levels (Fig 1). Instantaneous transitions were allowed between adjacent AMD states at any time, with 1 exception: regression from late AMD (level 50) to severe early AMD (level 40) was not allowed. Twelve transition intensities represent the hazard (instantaneous probability) of moving between adjacent states. Dependence of transition intensities on the exposure

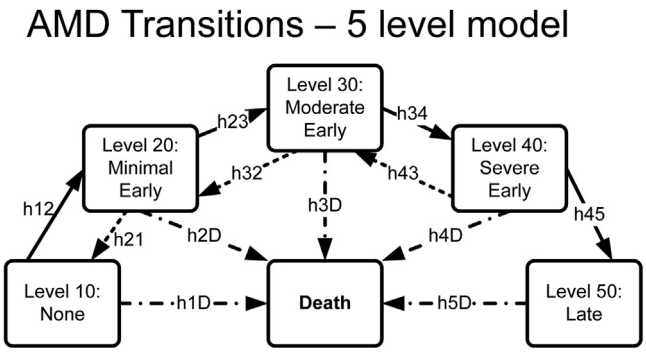

Figure 1. Transition diagram for the multi-state Markov model for the 5 levels of age-related macular degeneration (AMD) and an absorbing state of death. Boxes represent possible states for a person (worse eye) at any point in time. Changes between states over time (i.e., the outcomes) being simultaneously modeled are represented by arrows. The solid lines (h12, h23, h34, and h45) represent incidence and progression transitions for AMD. The dashed lines (h21, h32, h43) represent regression transitions. Transitions to death (h1D, h2D, h3D, h4D, and h5D) are allowed from all AMD states.

(ox-LDL) and covariates were specified using log-linear regression models with updating of time-varying factors. These models have previously been fit in this population.[32–34]

The MSM incorporates all available information on the history of disease progression into likelihood calculations. Current AMD severity was observed at intermittent study follow-up visits, which could be unevenly spaced, so exact transition times and the number of intermediate transitions were unobserved. Death times were available, but AMD severity at death was unknown. If subjects were alive at the end of follow-up, then final AMD severity was unknown. At study visits, the examination AMD state for the worse eye may not be known; for example, if the right eye had moderately severe early AMD (level 30) but the left eye was ungradable, the severity in the worse eye could be level 30, 40, or 50. The MSM models were set up to account for these unobserved possibilities. People were included in these analyses if they had at least 1 examination with both AMD status known in both eyes and ox-LDL measured. All subsequent visits with either AMD status (even if unknown in 1 eye) or ox-LDL values were also included. The final visit was based on survival status and date from annual follow-up with every participant. Deaths were identified by review of obituaries from local papers. Throughout this study, matches to the National Death Index have confirmed that the close ties this population has to the community means review of obituaries is quite complete and allows us to use the most up-to-date death information when cause-specific details are not needed.

Analyses were conducted in SAS version 9 and R[35] using the MSM package.[36] Covariate effects on transition intensities were summarized as hazard ratios, with 95% confidence intervals.

Results

The 50% random sample selected 2468 persons (8519 person-visits) for measurement, regardless of number of examinations, availability of sample, or AMD measures. Table 1 shows the baseline characteristics of those selected in the random sample to confirm no selection bias was present. The selected group had fewer women and slightly higher high-sensitivity C-reactive protein than the full population, but most characteristics were similar. Among the 8519 person-visits within selected individuals, samples were available in

Table 1. Comparison of Baseline Characteristics for Those Included (Selected for ELISA) and Excluded

Characteristic	Full Population		Selected (Random)		*P* Value*	In MSM Analysis		*P* Value†
	N	*Mean, % (SD)*	*N*	*Mean, % (SD)*	*vs. Full*	*N*	*Mean, % (SD)*	*vs. Selected*
Age (years)	4926	62.0 (11.2)	2440	62.1 (11.2)	0.83	1883	60.7 (10.7)	<0.001
Sex (% male)	4926	43.9	2440	41.3	<0.001	1883	41.5	0.63
BMI (kg/m^2)	4881	28.8 (5.4)	2419	28.8 (5.5)	0.80	1875	28.7 (5.5)	0.21
Systolic BP (mmHg)	4923	132.1 (20.5)	2439	132.2 (20.4)	0.99	1882	131.1 (19.7)	0.07
Diastolic BP (mmHg)	4923	77.3 (11.0)	2439	77.2 (11.0)	0.34	1882	77.6 (10.8)	0.64
Pulse pressure (mmHg)	4923	54.8 (17.8)	2439	55.0 (18.0)	0.51	1882	53.5 (17.0)	0.01
Hypertension	4917	50.6	2435	51.3	0.37	1880	49.6	0.76
Current smoker	4921	19.7	2438	19.6	0.94	1883	20.1	0.26
Cardiovascular disease	4855	15.1	2401	15.3	0.63	1858	12.8	0.002
Diabetes	4901	10.8	2422	11.2	0.30	1873	9.6	<0.001
eGFR (ml/min/1.73 m)	4880	79.5 (18.0)	2415	79.5 (18.1)	0.84	1874	80.6 (17.5)	0.31
hsCRP (mg/L)	4880	4.6 (10.1)	2415	4.9 (11.1)	0.01	1874	4.6 (10.7)	0.03
Cystatin-C (mg/L)	4639	0.9 (0.3)	2289	0.9 (0.3)	0.76	1778	0.9 (0.3)	0.01
Total serum cholesterol (mg/dL)	4906	233.6 (44.2)	2430	232.7 (44.8)	0.15	1879	233.1 (44.0)	0.07
Serum HDL cholesterol (mg/dL)	4903	52.0 (17.6)	2427	52.2 (18.0)	0.27	1878	53.0 (18.3)	<0.001
Lipid-lowering meds	4906	4.5	2431	4.4	0.82	1879	4.7	0.06
Statin meds	4904	0.5	2430	0.6	0.52	1879	0.5	0.41
Any cataract, worse eye	4513	30.0	2226	29.9	0.98	1776	26.5	0.16
Any AMD, worse eye	4500	22.6	2219	23.2	0.24	1844	22.5	0.86
Late AMD, worse eye	4499	1.6	2220	1.5	0.53	1847	1.1	0.11
High genetic risk ARMS2-CFH	4331	7.7	2153	7.7	0.90	1883	7.6	0.85

AMD = age-related macular degeneration; BMI = body mass index; BP = blood pressure; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; meds = medications; MSM = multi-state Markov; SD = standard deviation.
*Age-adjusted comparison of those selected for ELISA panel (among the full population).
†Age-adjusted comparison of those included in the final MSM models (among those selected for ELISA panel).

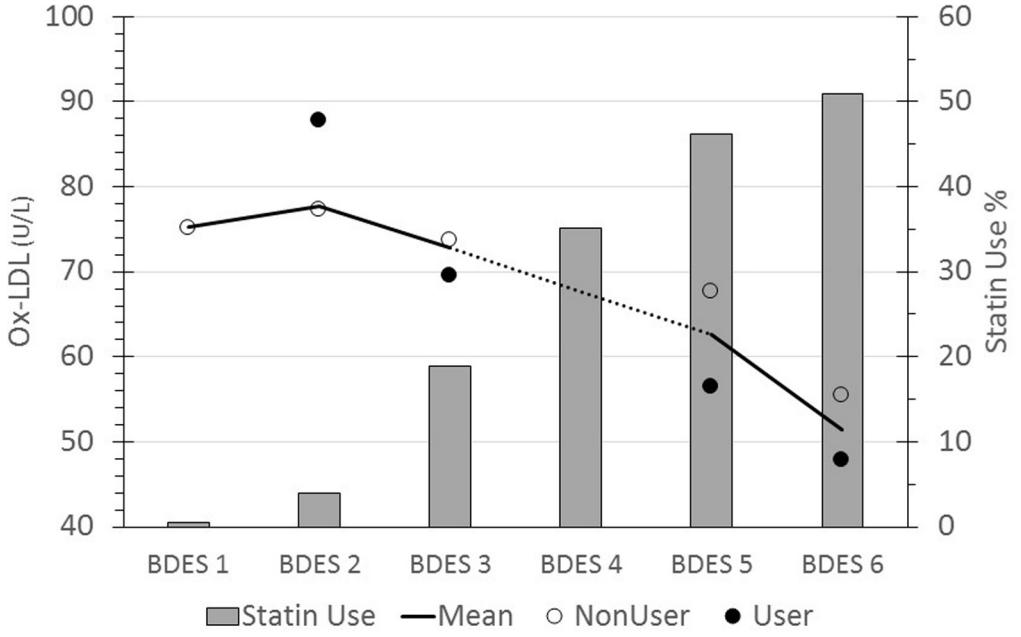

Figure 2. Distribution of oxidized low-density lipoprotein (ox-LDL) at each examination, including distribution by statin use. Line shows mean ox-LDL (U/L) at each examination. Circles show the mean among statin users (solid filled) and nonusers (open). The vertical bars show the percent of the population taking statins (see axis values to the right). The numbers with measurements are 1938 at BDES1, 1786 at BDES2, 1324 at BDES3, 868 at BDES4, and 515 at BDES5. No ox-LDL measures were available at BDES4 for any participant (N = 1196). BDES1 = Beaver Dam Eye Study baseline; BDES2–5 = Beaver Dam Eye Study follow-ups.

6431 from which ox-LDL was measured. The mean and standard deviation of ox-LDL at baseline (N = 1938) was 75.3 (23.1) U/L. The median was 73.0 U/L. Ox-LDL was normally distributed in the population at baseline, with the mean shifting to the left at more recent examination phases (mean [median] at BDES6 is 51.4 [49] U/L) (Fig 2). Statin use has increased from <5% in first 2 examination phases to over 50% by the most recent examination phase. At each examination, ox-LDL was lower among statin users, but even among non–statin users, there remains a left shift, so statin use does not entirely explain the left shift in ox-LDL. Oxidized serum LDL was found to be correlated to several factors, including age, serum total cholesterol, history of statin use, eGFR, diabetes status, blood pressure, and BMI.

Oxidized LDL was imputed from earlier visits, but there were still 724 of the 8519 person-visits selected where there was no ox-LDL measurement that could be used or imputed. In addition, 420 person-visits did not have at least 1 eye gradable for AMD along with ox-LDL measures, and 789 did not have data for 1 of the ARMS2 or

Table 2. Observed State Transitions During Consecutive Visit Intervals*

	AMD Level at Start of Interval					
AMD Level at End of Interval	*No AMD (level 10) (N=4499) N (%)*	*Minimal Early AMD (level 20) (N=889) N (%)*	*Moderate Early AMD (level 30) (N=486) N (%)*	*Severe Early AMD (level 40) (N=106) N (%)*	*Late AMD (level 50) (N=167) N (%)*	*Unknown in at least 1 eye (N=439) N (%)*
10	2937 (65)	81 (9)	2 (<1)	0 (0)	NA	40 (9)
20	212 (5)	339 (38)	37 (8)	0 (0)	NA	12 (3)
30	88 (2)	120 (13)	147 (30)	6 (6)	NA	10 (2)
40	7 (<1)	15 (2)	41 (8)	23 (22)	NA	6 (1)
50	1 (<1)	6 (<1)	27 (6)	28 (26)	65 (39)	11 (3)
10–50	217 (5)	34 (4)	13 (3)	2 (2)	4 (2)	80 (18)
20–50	6 (<1)	15 (2)	3 (<1)	0 (0)	1 (<1)	10 (2)
30–50	4 (<1)	9 (1)	17 (3)	4 (4)	0 (0)	12 (3)
40–50	0 (0)	1 (<1)	3 (<1)	3 (3)	0 (0)	3 (<1)
Death	604 (13)	189 (21)	140 (29)	31 (29)	78 (47)	171 (39)
Alive (no examination)	423 (9)	80 (9)	56 (12)	9 (8)	19 (11)	84 (19)

AMD = age-related macular degeneration; NA = not applicable.
*Consecutive visit intervals are from 1 examination (START of interval) to the next examination or to the final censoring status (alive and no later examinations or dead). The median time between examinations is 5 years, but some intervals may be longer (if missing an interim examination).

CFH risk alleles. The 6586 person-visits included in the MSM analysis come from 1893 individuals. Comparison of the BDES1 characteristics for the individuals included in the analyses with those that drop out (among the 2468 selected people) is shown in the last 2 columns of Table 1. Those that drop from analyses are more likely to be older, have cardiovascular disease, have diabetes, and have lower serum HDL cholesterol. This is similar to differences often found for analyses in this population, because older and sicker individuals are less likely to have gradable images for AMD. Indeed, the characteristics for people included in this analysis are very similar to the characteristics for people from the full population that were included in previously published MSM analyses of this outcome.

Person-specific covariates (sex, ARMS2-CFH risk) and time-varying covariates (age), exposure (ox-LDL), and AMD status are included for each examination. A final status, dead or alive (based on date of death), is also included following the last known examination for a person. The 6586 person-visits include 1884 as the final visit for a person (1213 died after that visit, and 671 were still alive and not seen), 4502 in which the visits were from consecutive examinations (1211 BDES1 to BDES2, 1143 BDES2 to BDES3, 914 BDES3 to BDES4, 742 BDES4 to BDES5), and 200 that were from pairs of visits that had a missed interim examination. The length of the interval ranged from a few days to 29 years, but most of the time (quartiles Q1 to Q3) was between 4.5 and 5.5 years, with a median of 5 years between records. The distribution of AMD levels for these pairs of visits is shown in Table 2. Among the 4702 person-visits with pairs of examinations, both visits had gradable AMD for 4182 person-visits; the remainder had at least partial AMD information. There were 308 (9%) with AMD (levels 20−50) at the follow-up visit among the 3245 without AMD (level 10) at the start of the interval. Late AMD (level 50) was observed at follow-up in 62 (2%) of the 4117 (3245 + 561 + 254 + 57) person-visits without late AMD at the start of the interval. The grading represents what is seen and does not use information from other visits or eyes to guide the evaluation, so regression and improvement may represent true improvement (disappearance of small drusen) or variations in imaging where a lesion was no longer visible.

The results of the MSM model are shown in Table 3. The table shows the hazard ratio (HR) and 95% confidence interval (CI) for the associations of ox-LDL with each of the possible transitions (Fig 1) of AMD while adjusting for other covariates. The association is considered significant if the 95% CI does not include 1. The HR (95% CI) for incidence of any AMD (transition from level 10 to level 20) was 1.03 (0.98−1.09) per 10 U/L while the HR for incidence of late AMD (transition from level 40 to level 50) was 0.97 (0.86, 1.09) per 10 U/L. The only significant association of ox-LDL with AMD was for regression from level 40 to 30, which was only observed in 6 person-visits. Table 3 also shows the associations of the other covariates with the AMD transitions, as well as all the associations with the transitions to death, including the risks for death from each of the AMD levels. Age and CFH-ARMS2 risk alleles are associated with most AMD incidence and progression transitions, as well as death. Oxidized serum LDL was not associated with death. Additional models were considered with adjustment for factors associated with ox-LDL, such as statin use, renal function, smoking status, and BMI, but none of these factors showed evidence of being confounders of the ox-LDL and AMD associations, and they were not included in the final model. Analyses using quartiles of ox-LDL also showed no suggestion of a threshold effect from highest (or lowest) quartiles.

Table 3. Multi-State Markov Model Results for Oxidized Low-density Lipoprotein with 5-level Age-related Macular Degeneration Outcome (Worse Eye)

	Hazard Ratio and 95% Confidence Interval for MSM Transitions							
	Incidence of Any AMD	*Progression of AMD*		*Incidence of Late AMD*	*Disappearance of AMD*	*Regression of AMD*		
Factor	*(10 to 20)*	*(20 to 30)*	*(30 to 40)*	*(40 to 50)*	*(20 to 10)*	*(30 to 20)*	*(40 to 30)*	*Death*
ox-LDL (per 10 U/L)	1.03 (0.98–1.09)	0.98 (0.93–1.04)	0.98 (0.89–1.08)	0.97 (0.86–1.09)	0.99 (0.90–1.09)	1.04 (0.90–1.19)	0.67 (0.49–0.93)*	1.00 (0.97–1.02)
Age (per 5 yrs)	1.54 (1.45–1.65)*	1.36 (1.25–1.48)*	1.29 (1.08–1.54)*	1.30 (1.06–1.58)*	1.07 (0.93–1.23)	0.89 (0.73–1.07)	2.71 (1.24–5.94)*	1.75 (1.68–1.81)*
Sex (male)	1.01 (0.80–1.26)	0.81 (0.61–1.08)	0.68 (0.40–1.16)	1.27 (0.66–2.43)	1.22 (0.78–1.89)	0.69 (0.35–1.38)	5.58 (0.83–37.7)	1.42 (1.26–1.60)*
Gene risk: moderate vs. low†	1.39 (1.09–1.78)*	1.53 (1.13–2.06)*	1.59 (0.92–2.75)	1.65 (0.92–2.98)	0.75 (0.42–1.31)	0.48 (0.21–1.10)	2.17 (0.45–10.4)	1.00 (0.87–1.14)
Gene risk: high vs. low†	1.51 (1.00–2.29)*	1.97 (1.26–3.06)*	1.84 (1.03–3.29)*	3.28 (1.58–6.81)*	0.54 (0.17–1.75)	0.36 (0.08–1.68)	0.18 (0.00–21.5)	1.01 (0.81–1.27)
Visit	0.93 (0.85–1.01)	0.96 (0.86–1.07)	1.04 (0.84–1.29)	1.12 (0.87–1.44)	1.46 (1.22–1.75)*	1.10 (0.83–1.45)	1.20 (0.52–2.73)	0.91 (0.87–0.95)*
AMD: 20 vs. 10								0.90 (0.61–1.31)
AMD: 30 vs. 10								1.48 (1.17–1.87)*
AMD: 40 vs. 10								0.36 (0.07–1.89)
AMD: 50 vs. 10								1.19 (0.92–1.53)

AMD = age-related macular degeneration; MSM = multi-state Markov; ox-LDL = oxidized low-density lipoprotein.
*Significant association (95% Confidence Interval does not include 1).
†Gene risk is defined as low (0-1 risk alleles), moderate (2 risk alleles) and high (3-4 risk alleles).

Discussion

We did not find any evidence of statistically significant relationships of higher levels of ox-LDL cholesterol to the

incidence and progression of AMD in the BDES. Our findings are consistent with earlier findings from the BDES of a lack of relationships of 2 other oxidative stress biomarkers: serum 8-isoprostane, a biomarker of lipid oxidation; and total carbonyl content, a biomarker of protein oxidation to the incidence of early AMD.[37] To our knowledge, there are no other population-based data with which to compare our findings.

The RPE has been shown to be vulnerable to oxidative damage by radical-catalyzed lipid peroxidation.[38–40] The lack of an association may be due to oxidative stress not being related to the incidence or progression of early or late AMD or the fact that the 3 biomarkers (ox-LDL, serum 8-isoprostane, and total carbonyl content) do not reflect oxidative stress occurring at the cellular level at the RPE. In a study involving 77 patients with AMD and 75 control participants, plasma F2 isoprostane, an oxidative stress biomarker, was found not to be related to AMD after adjustment for age, sex, and smoking status.[41] The role of ox-LDL in the pathogenesis of AMD is not well understood. We had hypothesized that there would be a direct relationship of serum ox-LDL to the development and progression of AMD based on previous observations in studies of human RPE in tissue culture, which showed that exposure to ox-LDL, but not native LDL, resulted in apoptosis and RPE cell death. In other studies, immune complexes of ox-LDL have been shown to affect transcriptional responses of genes involved in inflammatory pathways, a mechanism hypothesized to be involved in the pathogenesis of AMD.[42] We are unaware of population-based studies that have examined differences in the relationship of native LDL to oxidized LDL and the incidence or worsening of AMD.

A strength of our study was the inclusion of standard protocols to measure AMD from fundus photographs during a 25-year period in a representative population-based study. Our measurement of ox-LDL several times over 25 years, in many of the individuals, provided us with a representative estimate of association over a lifetime. An advantage of using the MSM model is its ability to account for survival as well as all stages of AMD. The incidence of late AMD was low, but accumulating all cases over 25 years maximizes our power. Although failure to reject the null hypothesis does not necessarily provide strong evidence in support of a negative finding (possibly reflecting a lack of power to detect meaningful differences), the narrow confidence intervals in the current study, which exclude any differences of clinical significance, do, in fact, provide strong support for a true lack of association.

In conclusion, these data do not support a role of oxidative stress as measured using serum levels of ox-LDL in the pathogenesis of AMD. There are likely to be many mechanisms involved in the development of this complex multifactorial disease.

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Footnotes and Financial Disclosures

Originally received: September 10, 2018.
Final revision: November 26, 2018.
Accepted: December 11, 2018.
Available online: December 17, 2018. Manuscript no. 2018-2076.

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Financial Disclosure(s):
The author(s) have made the following disclosure(s): B.E.K.K. and R.K.: Supported by grant EY06594 from the National Institutes of Health, Bethesda, MD and an unrestricted grant from Research to Prevent Blindness, New York, NY. The sponsor or funding organization had no role in the design or conduct of this research.

HUMAN SUBJECTS: Human subjects were included in this study. Approval was granted by the institutional review board at the University of Wisconsin. Written informed consent was also granted by the institutional review board for the use and disclosure of protected health information, which was obtained from all subjects before being enrolled in the study and before each examination. The study was performed in accordance with the tenets of the Declaration of Helsinki and the Health Insurance Portability and Accountability Act.

No animal subjects were used in this study.

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Obtained funding: R Klein, BEK Klein

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Abbreviations and Acronyms:
AMD = age-related macular degeneration; **BDES** = Beaver Dam Eye Study; **BMI** = body mass index; **CI** = confidence interval; **eGFR** = estimated glomerular filtration rate; **HDL** = high-density lipoprotein; **HR** = hazard ratio; **LDL** = low-density lipoprotein; **MSM** = Multi-State Markov; **ox-LDL** = oxidized serum low-density lipoprotein; **RPE** = retinal pigment epithelium.

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