Original Investigation

Oxidized Low-Density Lipoprotein and the Incidence of Proliferative Diabetic Retinopathy and Clinically Significant Macular Edema Determined From Fundus Photographs

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IMPORTANCE Studies have shown oxidized low-density lipoprotein to be associated with the incidence of proliferative retinopathy and other complications of type 1 diabetes mellitus. Because low-risk interventions are available to modify oxidized low-density lipoprotein, it is important to examine the relationships between this factor and the incidence of proliferative retinopathy and of macular edema, 2 important causes of visual impairment in people with type 1 diabetes.

OBJECTIVE To determine the association of oxidized low-density lipoprotein with the worsening of diabetic retinopathy and the incidence of proliferative retinopathy and of macular edema.

DESIGN, SETTING, AND PARTICIPANTS Of 996 participants with type 1 diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy, 730 were examined up to 4 times (1990-1992, 1994-1996, 2005-2007, and 2012-2014) over 24 years and had assays of oxidized low-density lipoprotein and fundus photographs gradable for diabetic retinopathy and macular edema. Analyses started July 2014 and ended February 2015.

MAIN OUTCOMES AND MEASURES Worsening of diabetic retinopathy, incidence of proliferative diabetic retinopathy, and incidence of macular edema as assessed via grading of color stereo film fundus photographs. The levels of oxidized low-density lipoprotein collected from serum samples at the time of each examination were measured in 2013 and 2014 from frozen serum.

RESULTS The cohort at baseline had a mean (SD) level of oxidized low-density lipoprotein of 30.0 (8.5) U/L. While adjusting for duration of diabetes, glycated hemoglobin A_{1c} level, and other factors, we found that neither the level of oxidized low-density lipoprotein at the beginning of a period nor the change in it over a certain period was associated with the incidence of proliferative diabetic retinopathy (hazard ratio [HR], 1.11 [95% CI, 0.91-1.35], $P = .30$; odds ratio [OR], 1.77 [95% CI, 0.99-3.17], $P = .06$), the incidence of macular edema (HR, 1.04 [95% CI, 0.83-1.29], $P = .74$; OR, 1.08 [95% CI, 0.44-2.61], $P = .87$), or the worsening of diabetic retinopathy (HR, 0.94 [95% CI, 0.83-1.07], $P = .34$; OR, 1.32 [95% CI, 0.83-2.09], $P = .24$).

CONCLUSIONS AND RELEVANCE Our findings do not provide evidence for a relationship between increasing levels of serum oxidized low-density lipoprotein and the incidence of macular edema or the worsening of diabetic retinopathy in persons with type 1 diabetes. The potential increase in the HR for incident proliferative retinopathy, with an increase in oxidized low-density lipoprotein level over the preceding period, warrants further investigation of this relationship.

JAMA Ophthalmol. 2015;133(9):1054-1061. doi:10.1001/jamaophthalmol.2015.2239
Published online July 16, 2015.

Supplemental content at jamaophthalmology.com

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The generation of oxidizing compounds is physiologically normal and is an important component of the body's inflammation and tissue-repair processes.[1,2] Oxidizing compounds represent part of the normal mechanism of defense against invading microorganisms and malignant cells, and they form during tissue healing and remodeling. Oxidative stress may occur when oxidizing compounds accumulate owing to the tissue's inability to metabolize them. Oxidative stress in persons with type 1 diabetes mellitus has been attributed to hyperglycemia, nonenzymatic protein glycation, and the decreased presence of antioxidants, resulting in less removal of reactive oxygen species.[3] This may lead to the peroxidation of low-density lipoprotein (LDL), which has been hypothesized to cause the death of retinal vessel pericytes, endothelial cells, and retinal cells of Müller, resulting in exacerbation of diabetic retinopathy (DR).[4]

Few long-term cohort studies have examined the association of serum oxidized LDL (ox-LDL) with the incidence and worsening of DR in persons with diabetes.[4] In our report, we examine relationships of serum ox-LDL level with the incidence of proliferative DR (PDR), the incidence of diabetic macular edema (DME), and the worsening of DR by 2 or more steps in participants with type 1 diabetes in the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR).

At a Glance

- We examined the relationship between the level of oxidized low-density lipoprotein and the incidence of proliferative retinopathy and of macular edema in type 1 diabetes.
- We found an increase in risk for incident proliferative diabetic retinopathy with an increase in the level of oxidized low-density lipoprotein over the preceding period that warrants further investigation.
- We did not find evidence that the level of oxidized low-density lipoprotein is associated with the incidence of macular edema independent of other risk factors.

Methods

Study Population

Detailed descriptions of the WESDR cohort, participation statistics, and reasons for nonparticipation have appeared elsewhere.[5-11] In brief, the WESDR identified 1210 persons with type 1 diabetes living and receiving care in an 11-county area of south central Wisconsin in 1979-1980. A group of 996 persons 3 to 79 years of age with 1 to 59 years of type 1 diabetes was first examined in 1980-1982. These persons participated in up to 6 follow-up examinations in 1984-1986 (n = 903), 1990-1992 (n = 816), 1994-1996 (n = 667), 2000-2001 (n = 567), 2005-2007 (n = 520), and 2012-2014 (n = 414).[5-11] No stereoscopic fundus photographs were taken at the 2000-2001 examination, and data from that examination were not used in the analyses. No individuals with a history of kidney or pancreas transplant or renal dialysis were included in the analyses. The tenets of the Declaration of Helsinki were followed, and institutional review board approval was obtained from the University of Wisconsin in Madison. Written informed consent was obtained from each participant.

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 lipid-lowering medications.

Laboratory Procedures

Nonfasting blood samples were collected, processed within 1 hour of collection, and frozen at −80°C beginning at the third examination in 1990-1992 and through 4 follow-up examinations. The ox-LDL level was measured in these samples; thus, the third examination serves as the "baseline" examination for the purposes of this analysis. A total of 2301 samples of serum were collected from 730 participants at up to 4 examinations between 1990-1992 and 2012-2014. During the 1990-1992, 1994-1996, 2005-2007, and 2012-2014 examinations, serum samples obtained from 638, 501, 425, and 289 participants, respectively, were frozen. At the time of the 2012-2014 examination, all of these frozen serum samples were sent to the University of Minnesota Advanced Research and Diagnostic Laboratory in Minneapolis for analysis. These serum samples were measured for ox-LDL levels in 2 batches, one in 2013 and the other in 2014. The ox-LDL level was measured by use of an enzyme-linked immunosorbent assay (Mercodia, Uppsala, Sweden). The intensity of the color was measured on a SpectraMax spectrophotometer (Molecular Device). The interassay coefficient of variation reported in the kit insert is from 4.0% to 6.2%. A set of quality-control samples accompanied each batch to ensure comparability of the ox-LDL levels between the batches. The methods used to measure the levels of other lipids, glycated hemoglobin A_{1c} (HbA1c), serum creatinine, microalbuminuria, and gross proteinuria can be found in Klein et al.[12]

Assessment of Presence and Severity of DR

After the participants' pupils were dilated, 30° stereoscopic color film photographs of the Early Treatment Diabetic Retinopathy Study 7 standard fields of the retina were obtained for each eye at each examination, except the 2000-2001 examination. The Airlie House classification system was used for grading retinopathy, and a modified concatenated 15-step Early Treatment Diabetic Retinopathy Study severity scale was used to define the presence and severity of DR in which the eye with more severe retinopathy was given more weight.[13,14] An increase in DR severity by 2 or more steps for a person defined worsening. Incidence of PDR was defined as developing severity level 60 or greater in 1 or both eyes in a person who did not have PDR in either eye at the beginning of a study interval. Diabetic macular edema was defined as the presence of retinal thickening involving the macula.

Definitions

Age was defined as the age at the time of each examination. Age at diagnosis of diabetes was defined as the age at the time

the diagnosis was first recorded by a physician in the participant's medical record or in a hospital record. The duration of diabetes was defined as the period between the age at diagnosis and the age at the specific 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.[15]

A participant was classified as a never smoker if he or she had smoked fewer than 100 cigarettes in his or her lifetime, an ex-smoker if he or she smoked 100 or more cigarettes in his or her lifetime but had stopped smoking before the examination, and a current smoker if he or she had not stopped smoking. Body mass index (BMI) was defined as the participant's weight in kilograms divided by the height in meters squared. Proteinuria was defined as a urine protein concentration of 30 mg/dL or more as measured by Labstix (Ames).

Statistical Methods

While adjusting for duration of diabetes, HbA_{1c} level, statin use, sex, proteinuria, smoking status, BMI, systolic blood pressure, WESDR visit, and the DR severity level at the time the serum sample of ox-LDL was obtained, we examined the association of the ox-LDL levels at the beginning of 3 periods (1990-1992, 1994-1996, and 2005-2007) with the incidence of PDR, the incidence of DME, and 2 or more steps of worsening of DR at the end of each period (1994-1996, 2005-2007, and 2012-2014). Data from the 3 periods were analyzed together using models that accounted for the varying lengths of time between examinations (4 years between the 1990-1992 and 1994-1996 examinations, 11 years between the 1994-1996 and 2005-2007 examinations, and 7 years between the 2005-2007 and 2012-2014 examinations). Duration of diabetes was used as the time scale, and the baseline hazard ratio (HR) was assumed to be piecewise constant with duration of diabetes categorized as less than 20 years, 20 to 29 years, and more than 30 years.

The association of an increase in ox-LDL level during the 4-year period between the 1990-1992 and 1994-1996 examinations with the subsequent 11-year incidence of PDR and of DME and the worsening of DR between the 1994-1996 and 2005-2007 examinations was modeled using logistic regression in SAS version 9.1 (SAS Institute). Models were adjusted for sex and duration of diabetes, as well as changes in HbA_{1c} level, statin use, proteinuria, smoking status, BMI, systolic blood pressure, and DR severity from the beginning to the end of the 4-year period.

Deming regression[16] was used to evaluate the comparability of the ox-LDL levels obtained from the quality-control samples between the first and second batches sent to the laboratory. We found that the ox-LDL levels in the quality-control samples in the first batch were not comparable to those in the second batch ($P < .05$). Based on these results, we transformed the levels for all the samples in the second batch to be comparable to those in the first batch. The distribution of baseline ox-LDL levels, after the transformation of the samples obtained from participants in the second batch, is presented in eFigure 1 in the Supplement.

Results

Participant Characteristics

There were 813 persons (1886 person-intervals) who participated in the WESDR examinations in 1990-1992, 1994-1996, and/or 2005-2007. Of the 1886 person-intervals, 322 were excluded because frozen serum samples were unavailable to measure the ox-LDL level; 709 were excluded because the participant was not at risk for any of the outcomes of interest or because there was missing data on DR severity, DME, or PDR status; and 16 were excluded because of missing data on covariates. After these person-intervals were excluded, 428 participants remained eligible, and they contributed 832 person-intervals to the analyses (728 person-intervals for worsening of DR and incidence of PDR and 720 person-intervals for incidence of DME). At the baseline WESDR examination, the participants included in the analyses were more likely to be younger and, after adjustment for age and sex, were more likely to have a shorter duration of diabetes, a lower HbA_{1c} level, a lower ox-LDL level, a lower serum total cholesterol level, and lower systolic blood pressure, and were less likely to have proteinuria, prevalent PDR, or DME compared with those who were excluded (**Table 1**).

Distribution of Oxidized LDL Levels and Associated Risk Factors

The mean (SD) ox-LDL level was 30.0 (8.5) U/L. We found significant inverse trends of age, duration of diabetes, and history of using statins. We found positive associations of HbA_{1c} level, systolic blood pressure, BMI, presence of proteinuria, and presence of PDR with increasing quartile ranges of ox-LDL level (**Table 2**). The results were similar when the ox-LDL level was treated continuously (data not shown). We did not find any associations of sex, systolic blood pressure, and history of current smoking with ox-LDL levels.

Incidence of PDR and DME and Worsening of DR

Among the 428 participants studied, DR worsened in 227 of 356 participants at risk (63.8%) by 2 or more steps, 90 of 356 participants at risk (25.3%) developed PDR, and 64 of 382 participants at risk (16.8%) developed DME over the 22-year period. While adjusting only for duration of diabetes, ox-LDL level was associated with a 53% increase in the hazard of incident PDR (HR per 10 U/L = 1.53 [95% CI, 1.30-1.79]; $P < .001$) (**Table 3**). Oxidized LDL level was also associated with the incidence of DME (HR per 10 U/L = 1.27 [95% CI, 1.05-1.54]; $P = .01$) and worsening of DR (HR per 10 U/L = 1.13 [95% CI, 1.02-1.26]; $P = .02$). Oxidized LDL level remained significantly related to the incidence of PDR but not the incidence of DME or the worsening of DR after inclusion of HbA_{1c} level in the model (Table 3). After further adjustment for sex, statin use, examination phase, proteinuria, smoking status, BMI, and baseline DR severity, ox-LDL level was no longer associated with the incidence of PDR (HR, 1.11 [95% CI, 0.91-1.35]; $P = .30$), the incidence of DME (HR, 1.04 [95% CI, 0.83-1.29]; $P = .74$), or the worsening of DR (HR, 0.94 [95% CI, 0.83-1.07]; $P = .34$). Adjusting for other factors, we did not find significant relation-

Table 1. Characteristics of Participants at the Baseline Examination Included and Excluded From Analyses of Incidence, Wisconsin Epidemiologic Study of Diabetic Retinopathy, 1990-1992

Covariate	Participants		P Value[a]
	Included (n = 428)	Excluded (n = 385)	
Age, mean (SD), y	35.7 (11.1)	38.5 (12.1)	<.001[b]
Duration of diabetes, mean (SD), y	21.1 (8.3)	25.0 (10.0)	<.001
HbA_{1c}, mean (SD), %	9.1 (1.5)	9.5 (1.8)	<.001
Oxidized LDL, mean (SD), U/L	32.1 (10.3)	35.9 (12.1)	<.001
BMI, mean (SD)	25.8 (3.8)	25.8 (4.4)	.84
Serum total cholesterol, mean (SD), mg/dL	190.1 (42.4)	205.5 (47.0)	<.001
SBP, mean (SD), mm Hg	123.5 (16.3)	129.1 (21.3)	.003
Male sex, %	50.6	48.0	.46[c]
Currently smoking, %	40.1	44.0	.27
Proteinuria present, %	20.3	44.0	<.001
Currently using statins, %	1.5	2.1	.54
Prevalent PDR, %	17.0	64.5	<.001
Prevalent macular edema, %	6.3	43.3	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HbA_{1c}, glycated hemoglobin A_{1c}; LDL, low-density lipoprotein; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

SI conversion factors: To convert HbA_{1c} to proportion of total hemoglobin, multiply by 0.01; and to convert total cholesterol to millimoles per liter, multiply by 0.0259.

[a] Adjusted for age and sex except where noted.

[b] Adjusted for sex.

[c] Adjusted for age.

Table 2. Unadjusted Associations of Covariates With Oxidized Low-Density Lipoprotein Levels by Quartiles

Covariate	Quartile 1 (9.3-24.5 U/L)	Quartile 2 (24.6-28.2 U/L)	P Value[a]	Quartile 3 (28.3-34.2 U/L)	P Value[a]	Quartile 4 (34.3-90.4 U/L)	P Value[a]	Overall P Value[b]
Age, mean (SD), y	40.3 (12.1)	40.4 (11.5)	.15	37.4 (10.9)	.10	39.9 (11.3)	.002	.006
Duration of diabetes, mean (SD), y	26.2 (10.0)	24.8 (9.3)	.22	24.0 (9.0)	.11	24.4 (8.1)	.002	.006
HbA_{1c}, mean (SD), %	8.2 (1.4)	8.4 (1.5)	.35	8.6 (1.5)	<.001	9.2 (1.6)	<.001	<.001
SBP, mean (SD), mm Hg	123.3 (17.6)	122.9 (15.0)	.64	123.3 (15.7)	.66	128.1 (17.9)	.02	.05
BMI, mean (SD)	25.8 (4.1)	26.7 (4.0)	.20	26.4 (4.2)	.23	27.5 (4.7)	<.001	<.001
Male sex, %	44.6	52.4	.15	52.7	.21	52.4	.11	.12
Nephropathy present, %	8.5	11.5	.64	13.0	.29	30.6	<.001	<.001
Currently using statins, %	14.2	10.1	.33	6.8	.05	7.8	.41	.17
Proteinuria present, %	8.5	11.5	.64	13.0	.29	30.6	<.001	<.001
Currently smoking, %	36.0	41.4	.11	39.1	.57	41.8	.50	.61
Prevalent, %								
PDR	7.1	11.1	.12	13.1	.03	15.5	.02	.02
DME	6.2	7.7	.85	5.3	.54	10.8	.68	.82
Incident, %								
PDR	9.2	7.6	.63	12.5	.31	20.8	.002	<.001
DME	7.2	6.5	.79	6.9	.90	15.6	.02	.02
Worsening of DR, %	36.9	37.0	.79	41.5	.40	44.5	.16	.10

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DME, diabetic macular edema; DR, diabetic retinopathy; HbA_{1c}, glycated hemoglobin A_{1c}; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

SI conversion factor: To convert HbA_{1c} to proportion of total hemoglobin, multiply by 0.01.

[a] Compared with quartile 1.

[b] For trend over quartiles.

ships between ox-LDL levels modeled as quadratic terms or in quartiles and the incidence of PDR or DME or the worsening of DR (data not shown).

Change in ox-LDL Level and Incidence of PDR and DME and Worsening of DR

There were 318 persons who participated in examinations in 1990-1992, 1994-1996, and 2005-2007. Of these 318 participants, 78 were excluded because they did not have serum samples for analysis or were missing laboratory data on ox-LDL levels at either the 1990-1992 or 1994-1996 examination, 74 were excluded because their retinal photographs taken at the 1994-1996 and 2005-2007 examinations were not gradable or because they were not at risk for any of the outcomes of interest, and 15 were excluded because they were missing data on covariates. After these 167 participants were excluded, 151 participants remained eligible, with 144 contributing to analyses of the 11-year worsening and incidence of PDR

Table 3. Associations of Oxidized Low-Density Lipoprotein Level With the Incidence of PDR and DME and Worsening of DR[a]

	Model 1		Model 2		Model 3		Model 4	
Outcome	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Incidence								
PDR	1.53 (1.30-1.79)	<.001	1.27 (1.07-1.52)	.007	1.18 (0.99-1.40)	.06	1.11 (0.91-1.35)	.30
DME	1.27 (1.05-1.54)	.01	1.12 (0.91-1.38)	.27	1.07 (0.88-1.32)	.49	1.04 (0.83-1.29)	.74
Worsening of DR	1.13 (1.02-1.26)	.02	1.01 (0.90-1.14)	.81	1.02 (0.91-1.14)	.77	0.94 (0.83-1.07)	.33

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy.

[a] Model 1 adjusts for duration of diabetes (as the time scale). Model 2 additionally adjusts for glycated hemoglobin A_{1c} level. Model 3 further adjusts for DR severity level (categorized as none, mild/moderate, and severe nonproliferative DR). Model 4 additionally adjusts for sex, study visit, body mass index, presence of proteinuria, statin use, systolic blood pressure, and smoking habits.

Table 4. Characteristics of Participants at the Baseline Examination Included and Excluded From Analyses of Change, Wisconsin Epidemiologic Study of Diabetic Retinopathy, 1990-1992

	Participants		
Covariate	Included (n = 151)	Excluded (n = 167)	*P* Value[a]
Age, mean (SD), y	34.0 (8.6)	34.6 (8.9)	.21[b]
Duration of diabetes, mean (SD), y	18.9 (6.2)	21.2 (6.5)	.001
HbA_{1c}, mean (SD), %	9.0 (1.4)	9.4 (1.6)	.01
Oxidized LDL, mean (SD), U/L	30.0 (8.5)	32.3 (9.1)	.03
BMI, mean (SD)	26.0 (3.5)	25.7 (3.8)	.38
Serum total cholesterol, mean (SD), mg/dL	182.3 (32.8)	193.3 (43.0)	.02
SBP, mean (SD), mm Hg	121.4 (14.2)	124.4 (16.4)	.04
Male sex, %	53.3	46.4	.22[c]
Currently smoking, %	35.5	30.1	.27
Proteinuria present, %	12.5	26.1	.003
Currently using statins, %	0.7	3.0	.16
Prevalent PDR, %	3.3	56.6	<.001
Prevalent macular edema, %	4.0	26.6	<.001

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HbA_{1c}, glycated hemoglobin A_{1c}; LDL, low-density lipoprotein; PDR, proliferative diabetic retinopathy; SBP, systolic blood pressure.

SI conversion factors: To convert HbA_{1c} to proportion of total hemoglobin, multiply by 0.01; and to convert total cholesterol to millimoles per liter, multiply by 0.0259.

[a] Adjusted for age and sex except where noted.

[b] Adjusted for sex.

[c] Adjusted for age.

and 123 contributing to the analyses of the 11-year incidence of DME. At the baseline examination, the participants included in the analyses were more likely to have a shorter duration of diabetes, a lower HbA_{1c} level, a lower serum total cholesterol level, a lower ox-LDL level, lower systolic blood pressure, and less prevalent proteinuria, PDR, or DME compared with those excluded after adjusting for age and sex (**Table 4**).

The distribution of change in ox-LDL level between the baseline and 4-year follow-up examinations is shown in eFigure 2 in the Supplement. Change in ox-LDL level was slightly skewed to the right with a mean (SD) of 1 (7.0) U/L.

Among the 151 participants remaining in our study, DR worsened in 49 of 144 participants at risk (34.0%) by 2 or more steps, 21 of 144 participants at risk (14.6%) developed PDR, and 4 of 123 participants at risk (3.3%) developed DME over the subsequent 11-year period. Adjusting for age and sex, we found that the change in ox-LDL level was marginally associated with the incidence of PDR (odds ratio [OR], 1.63 [95% CI, 0.96-2.76]; $P = .07$) but not with the incidence of DME (OR, 1.20 [95% CI, 0.47-3.05]; $P = .70$) or the worsening of DR (OR, 1.40 [95% CI, 0.91-2.14]; $P = .12$). After further adjustment for change in HbA_{1c} level, history of statin use, proteinuria, smoking status, BMI, and DR level over the same period, there remained a marginal association of a 77% higher subsequent 11-year incidence of PDR with a 10-U/L increase in the ox-LDL level between the 1990-1992 and 1994-1996 examinations (OR, 1.77 [95% CI, 0.99-3.17]; $P = .06$) (**Table 5**). Change in ox-LDL level was not associated with the worsening of DR (OR, 1.32 [95% CI, 0.83-2.09]; $P = .24$) or the incidence of DME (OR, 1.08 [95% CI, 0.44-2.61]; $P = .87$).

Discussion

In the WESDR, while adjusting for duration of diabetes and other covariates, we found no significant relationships between serum ox-LDL level and the incidence of PDR, incidence of DME, or worsening of DR. To our knowledge, there are no cohort studies that have measured the relationships between change in serum ox-LDL level and the subsequent incidence of PDR and DME and the worsening of DR to which WESDR findings can be compared. The Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study[17] used a different measure of ox-LDL that is found in immune complexes, which is a result of the formation of autoantibodies due to the activation of the T cells by ox-LDL. In this study, while adjusting for HbA_{1c} level,

Table 5. Associations of Change in ox-LDL Level Between Baseline and 4-Year Follow-up Examination With 11-Year Incidence of PDR and DME and Worsening of DR

	Model 1[a]		Model 2[b]	
Outcome	OR (95% CI)[c]	*P* Value	OR (95% CI)[c]	*P* Value
Incidence				
PDR	1.63 (0.96-2.76)	.07	1.77 (0.99-3.17)	.06
DME	1.20 (0.47-3.05)	.70	1.08 (0.44-2.61)	.87
Worsening of DR	1.40 (0.91-2.14)	.12	1.32 (0.83-2.09)	.24

Abbreviations: DME, diabetic macular edema; DR, diabetic retinopathy; OR, odds ratio; ox-LDL, oxidized low-density lipoprotein; PDR, proliferative diabetic retinopathy.

[a] Adjusted for duration of diabetes.

[b] Adjusted for duration of diabetes, sex, and change in glycated hemoglobin A_{1c} level, body mass index, systolic blood pressure, smoking habits, presence of proteinuria, statin use, and DR severity level (categorized as none, mild/moderate, and severe nonproliferative DR).

[c] Per 10-U/L change in ox-LDL level.

DR severity, albumin excretion rate, and duration of diabetes, Lopes-Virella et al[17] found the serum immune complex ox-LDL level to be related to the incidence of PDR over a 16-year period in persons with no DR at baseline (HR per 1 SD = 2.17 [95% CI, 1.19-3.98]; $P = .01$) but not in persons with DR at baseline. They hypothesized that a higher serum immune complex ox-LDL level was more likely to have an effect earlier in the course of diabetes, prior to the onset of DR, through its damaging effect on the retinal pericytes, the inducement of leukostasis in the small retinal capillaries, and the stimulation of growth factors—all pathogenetic processes thought to be associated with the incidence of early DR.

Little is known about their potential roles in the pathogenesis of DR, but oxidized lipoproteins have been shown to increase the risk of atherosclerosis by promoting lipid accumulation in macrophages, which induces their transformation into foam cells. It has been shown that ox-LDL may precipitate an autoimmune reaction, producing immune complexes and promoting a proinflammatory environment.[18] Although ox-LDL immune complexes are upstream of ox-LDL, their correlation has been found to be modest but significant ($r = 0.313$; $P < .005$).[19] Oxidized LDL and ox-LDL immune complex are 2 different but related exposure variables, similar to LDL and ox-LDL, or to LDL and small LDL. These differences in ox-LDL and the ox-LDL immune complex may involve differences in the early transcriptional responses of genes involved with inflammation, a hypothesized mechanism in the pathogenesis of DR.[20] To our knowledge, there have been no direct comparisons in a cohort of persons with diabetes on the effect of ox-LDL and the ox-LDL immune complex on the risk of the worsening of DR and the incidence of DME or PDR.

A beneficial effect of antioxidants (eg, nicanartine, vitamin E, and lipoic acid) on DR lesions in diabetic animals has been found that suggests the possible role played by oxidative stress in the pathogenesis of DR.[21-23] Other studies[24-30] have hypothesized that oxidative stress in persons with type 1 diabetes is involved in the pathogenesis of not only DR but also diabetic nephropathy, myocardial infarction, and cognitive dysfunction.

Further support for the role of ox-LDL in the pathogenesis of DR comes from both the Action to Control Cardiovascular Risk in Diabetes Eye Study[31] and the Fenofibrate Intervention and Event Lowering in Diabetes study.[32] Both randomized controlled clinical trials reported a protective effect of fenofibrate on the worsening of DR. In their review, Yu and Lyons[33] summarized the possible mechanisms for the beneficial effect of fenofibrate, including its lowering of plasma ox-LDL levels,[34] its modulation of the oxidized low density lipoprotein (lectin-like) receptor 1 (*LOX-1*, the scavenger receptor for ox-LDL),[35] and its attenuation of the cellular effects of ox-LDL.[36] Yu and Lyons[33] had hypothesized that this effect of ox-LDL was due to the accumulation of intraretinal ox-LDL as a result of the breakdown of the blood-retinal barrier resulting in the death of retinal pericytes, endothelial cells, and cells of Müller.

Although there are many strengths to our study, including the use of similar standardized protocols to assess the incidence of PDR and of DME, there are also limitations. First, serum levels of ox-LDL may not reflect levels of ox-LDL in retinal tissue. It is presumed that the serum ox-LDL levels reflect similar levels of ox-LDL in the retinal tissue, but this may not be the case. Second, the role of oxidative processes cannot be separated from the effects of lipids themselves on the development and progression of retinopathy. Moreover, we do not know whether high lipid levels, irrespective of ox-LDL levels, are causing the changes in eyes that developed PDR. When we replaced ox-LDL with non–high-density lipoprotein cholesterol in the model, we found no evidence of a relationship (R.K., unpublished data, October 16, 2014). We did not find a relationship of serum non–high-density lipoprotein cholesterol to incident PDR.[37] Another limitation is that we do not know whether serum ox-LDL levels remained stable over the long period between being frozen and being measured. Oxidized LDL is stable for at least 3 years if stored at −70°C or below.[38] Selective survival may have affected the results. In the WESDR, while adjusting for other factors such as duration of diabetes and HbA_{1c} level, we found that persons with PDR with ox-LDL levels in the highest quartile were 8% more likely (HR, 1.08 [95% CI, 1.04-1.13]) to die sooner than persons with ox-LDL levels in the lowest quartile. A lack of power, as indicated by the wide 95% CIs obtained from model results, may have also limited our ability to be confident about finding or not finding associations.

Conclusions

In summary, we did not find evidence to support our hypothesis that serum levels of ox-LDL are associated with the incidence of DME and the worsening of DR independent of other risk factors. The increase in the HR for incident PDR, with an increase in the ox-LDL level over the preceding period, warrants further investigation. The limited power in our analysis and the selection biases (with regard to those at higher risk of worsening disease being less likely to be included in the analyses) must be considered.

ARTICLE INFORMATION

Submitted for Publication: March 16, 2015; final revision received May 29, 2015; accepted May 29, 2015.

Published Online: July 16, 2015. doi:10.1001/jamaophthalmol.2015.2239.

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Obtained funding: R. Klein, Cruickshanks, B. E. K. Klein.
Administrative, technical, or material support: Paterson, B. E. K. Klein.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This research was supported by grant EY016379 from the National Institutes of Health (Drs R. Klein and B. E. K. Klein) and an unrestricted grant from Research to Prevent Blindness, New York, New York.

Role of the Funder/Sponsor: The funding organizations had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Disclaimer: The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Eye Institute or the National Institutes of Health.

Previous Presentation: An abstract of this paper was presented at the Annual Meeting of the Association for Research and Vision in Ophthalmology; May 7, 2014; Orlando, Florida.

Additional Contributions: Heidi M. G. Christian, BA, and Erin L. Syth, MS, of the Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, assisted with the technical editing and preparation of the manuscript. They received no additional compensation beyond their normal wages as employees of the University of Wisconsin for their assistance.

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