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RETINAL DISORDERS

# Association of serum lipid levels with retinal hard exudate area in African Americans with type 2 diabetes

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**Abstract**
*Purpose* Previous studies have yielded conflicting results regarding whether serum lipid levels are associated with retinal hard exudates in diabetic retinopathy. The majority of studies have assessed hard exudates only as a dichotomous trait (presence vs. absence) and included limited numbers of African Americans (AA). The purpose of this study was to determine if there are any associations between serum lipid levels and hard exudates in AA with type 2 diabetes (T2D).

*Methods* 890 AA participants with T2D were enrolled from 5 sites. Macular fundus photographs were graded by masked ophthalmologist investigators. Hard exudate areas were measured using a semi-automated algorithm and ImageJ software. Multivariate regression models were used to determine the association between serum lipid levels and (1) presence of hard exudate and (2) area of hard exudate.
*Results* Presence of hard exudates was associated with higher total cholesterol [(odds ratio (OR) = 1.08, 95 % confidence interval (CI) 1.03–1.13, $P = 0.001$)] and higher low-density lipoprotein (LDL) cholesterol (OR = 1.08, 95 % CI 1.03–1.14, $P = 0.005$) in models controlling for other risk factors. Hard exudate area was also associated with both higher total and LDL cholesterol levels ($P = 0.04$ and 0.01, respectively) in multivariate models controlling for other risk factors.
*Conclusions* Higher total and LDL cholesterol were associated with the presence of hard exudates and a greater hard exudate area in AA with T2D. This information can be used to counsel diabetic patients regarding the importance of lipid control to decrease the risk of macular hard exudates.

**Keywords** Hard exudate area · Serum lipid levels · Type 2 diabetes · African Americans

This submission has not been published anywhere previously and is not simultaneously being considered for any other publication.

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## Introduction

Diabetic macular edema (DME) is an important cause of central vision loss in patients w,ith diabetes. Macular hard exudates are often seen in patients with DME and the exudates themselves, particularly when located subfoveally, can be a cause of permanent vision loss even after DME resolves [1]. Eventual disappearance of hard exudates is also not necessarily followed by improvement in visual acuity [1]. The failure of visual acuity to improve when exudation disappears could

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be due either to structural changes left at the site of the exudation, or to preceding neuronal degeneration, or both. This risk of permanent vision loss highlights the importance of hard exudate prevention and of identifying modifiable risk factors for hard exudates which could help guide counseling of patients with diabetes [1].

Previous studies have yielded inconsistent results regarding whether serum lipid levels are associated with retinal hard exudates. Among studies that have found an association between serum lipids and macular hard exudates, there is variability as to which lipid fractions are significantly associated. Table 1 summarizes the results of studies that have addressed this question thus far. These inconsistencies about which lipid subtypes, if any, affect hard exudate development could be due to differences in hard exudate assessment methods and heterogeneity of subjects with regards to age, gender, diabetes duration, diabetes type and ethnicity. Furthermore, most studies have examined only the dichotomous outcome of presence vs. absence of hard exudate rather than the continuous outcome of hard exudate area. In general examining a trait as a continuous variable vs. a dichotomous variable increases the power of an analysis [2–4]. For this particular question, examining a hard exudate as a continuous variable would allow us to determine associations not only with the presence of a hard exudate but also with the severity of the hard exudate.

There are a few diabetic retinopathy studies that have examined macular hard exudates and included significant numbers of AA participants [5–7]. To the best of our knowledge, the relationship between serum lipid levels and hard exudate area in an AA population has not been described. The purpose of this study is to determine if there are any associations between serum lipid levels and the presence and/or area of hard exudate in AA with T2D.

## Materials and methods

### Participants and fundus photograph grading

The Institutional Review Boards of the University of Mississippi Medical Center (UMMC), Massachusetts Eye and Ear Infirmary (MEEI), and Boston Medical Center (BMC) approved this study, and all participants gave written informed consent. All procedures conformed to the tenets of the Declaration of Helsinki.

AA participants with T2D were recruited between 2009 and 2013 from two studies: the African American Proliferative Diabetic Retinopathy (AAPDR) Study, which recruited participants from four different sites, and the Jackson Heart Study (JHS) which enrolled participants at one site (Table 2) [8, 9]. All participants self-identified as AA and had a known diagnosis of T2D by the 2003 American Diabetes Association (ADA) criteria [10] and/or by being on an anti-diabetic medication. We included patients who had laser therapy or anti-vascular endothelial growth factor (VEGF) intravitreal injections for diabetic retinopathy and/or clinically significant macular edema (CSME) in the past. Dilated, digital seven-standard field fundus photographs and a pair of macular images for stereoscopic viewing were obtained from both eyes using a Topcon TRC 50 DX camera (Topcon, Tokyo, Japan). Images were graded for degree of retinopathy and presence of CSME by two independent, masked ophthalmologist/investigators as previously described [8, 9]. They were also graded for the presence of focal laser scars.

For a subgroup of patients who also had macular optical coherence tomography (OCT) images available (Spectralis, Heidelberg, Germany), we recorded the central subfield mean thickness (CSMT) for each eye as previously described [11]. This was done in order to calculate the correlation of CSME identification on fundus photographs with center-involving DME identification from OCT images. Center-involving DME was defined as a CSMT >305 μm in women and >320 μm in men [12].

Images centered on the macula were also graded specifically for presence of hard exudates. Disagreements were arbitrated by a third masked ophthalmologist/investigator. Patients were excluded from the study if they had any ocular pathology that precluded adequate assessment of the fundus such as preretinal hemorrhage or a traction retinal detachment. The primary endpoint of the study was presence vs. absence of exudate. A participant was considered to have hard exudates if they were present in either or both eyes.

### Quantitative measurement of hard exudates

For patients who had hard exudates, we performed a secondary analysis for the endpoint hard exudate area. Because of variability in fundus photography magnification due to corneal curvature, axial length, refractive error and depth of focus, we used fixed retinal landmarks to determine the area of the fundus within which hard exudates would be quantified. For each participant with hard exudates, one fundus image centered on the macula was chosen for measurement. If both eyes had hard exudates, the right eye was used. The images were cropped to circles centered on the fovea with a radius equal to the distance between the fovea center and the temporal optic nerve margin. Hard exudate area was assessed with ImageJ software (National Institutes of Health, Bethesda, MD, USA; Fig. 1). The cropped images were split into three color channels and the green channel was used for the analysis because it highlights hard exudate pathology very well. We measured the cropped circle area in pixels. We measured the area of hard exudates using a semi-automated technique. First

**Table 1** Studies that have examined the association between serum lipid levels and hard exudates after adjusting for other diabetic retinopathy risk factors

| Study name | Ethnicities included/number of AA included | Diabetes type | Exudate assessment method | Association found with lipid fraction in multivariate analysis | | | |
|---|---|---|---|---|---|---|---|
| | | | | TC | LDL | HDL | TG |
| Klein et al. 1991 WESDR [19] | Multi-ethnic/unknown | IDDM | Three grades: no/one eye/both eyes | Y | N/A | N | N/A |
| Klein et al. 1991 WESDR [19] | Multi-ethnic/unknown | NIDDM | Three grades: no/one eye/both eyes | N | N/A | N | N/A |
| Chew et al. 1996 ETDRS [20] | Multi-ethnic/unknown | 1 and 2 | Six grades: none/questionable/definite/ obvious/moderate/severe | Y | Y | N | Y |
| Roy et al. 2001 [5] | AA/725 | 1 | Presence/absence | Y | Y | N | N |
| Klein et al. 2002 ARIC [6] | Blacks, whites/605 | 2 | Presence/absence | N | Y | N | N |
| Van Leiden et al. 2002 [21] | Caucasians/0 | 2 | Presence/absence | Y | Y | N | N |
| Klein et al. 2002 CHS [7] | Black and non-black/65 | 2 | Presence/absence | Y | Y | N/A | N/A |
| Miljanovic et al. 2004 DCCT [22] | Predominantly white/unknown | 1 | Six grades: none/questionable/definite/ obvious/moderate/severe | Y | Y | N | Y |
| Ucgun et al. 2007[38] | Turkish | 2 | Presence/absence | Y | Y | N | N |
| Sachdev et al. 2010 [25] | Indian | 2 | Modified Airlie House classification (three grades: none to severe) | Y | Y | N | Y |
| Idiculla et al. 2012 [23] | Indian | 2 | Presence/absence | Y | N | N | N |
| Sasaki et al. 2013 [24] | English-speaking but specific ethnicities not specified | 1 and 2 | Quantitative: Image J Qualitative: six grades: none/questionable/ definite/obvious/ moderate/severe | N | Y | N | Y |

*AA* African Americans, *ARIC* atherosclerosis risk in communities, *DCCT* diabetes control and complications Trial, *ETDRS* early treatment diabetic retinopathy Study, *HDL* high-density lipoprotein cholesterol, *LDL* low-density lipoprotein cholesterol, *IDDM* insulin-dependent diabetes mellitus, *NIDDM* non-insulin-dependent diabetes mellitus, *TC* total cholesterol, *TG* triglyceride, *WESDR* Wisconsin Epidemiologic Study of Diabetic Retinopathy

the "Maxentropy" function in ImageJ identified hard exudates in an automated fashion using an intensity threshold that was set to maximize capture of all possible hard exudates in the cropped circle. Then, two masked ophthalmologist/investigators evaluated the images and decided independently whether the automated function had missed or incorrectly identified hard exudates and manually corrected the images. Images graded by the two readers were compared and disagreements were arbitrated by a third ophthalmologist/investigator. The hard exudate area for each cropped image was then measured automatically in pixels using the measure function in ImageJ. We calculated a relative area of a hard exudate by dividing the hard exudate area by the total cropped image area.

### Intra- and inter-observer reliability assessment

To determine intra-observer reliability, 40 randomly sampled images were regraded for total hard exudate area by the same reader 3 months after the initial grading. Inter-observer agreement on total hard exudate area was assessed using all the images. Inter-observer agreement for presence of CSME was also evaluated.

### Covariates

One set of fasting lipid levels [total, low density lipoprotein (LDL), and high density lipoprotein (HDL) cholesterol and triglycerides] closest to the study visit but within one year of the study visit were obtained. The covariates examined in this study were age, gender, duration of diabetes, hemoglobin $A_{1c}$ ($HbA_{1C}$), systolic blood pressure, use of lipid-lowering medication, use of anti- hypertensive medication, Early Treatment Diabetic Retinopathy Study (ETDRS) diabetic retinopathy grade, presence of CSME and site of recruitment. Covariate data was collected by uniform methods at the study visit or from the medical record. Duration of diabetes was verified by review of the medical record. For each participant, a blood sample was sent for $HbA_{1c}$ measurement as part of the study. For the statistical analyses, duration of diabetes, $HbA_{1c}$, mean systolic blood pressure, serum fasting total cholesterol, HDL

**Table 2** Distribution of patients by study and sites

| Study name | Site name | Number of patients in study |
|---|---|---|
| African American Proliferative Diabetic Retinopathy Study | | |
| | UMMC | 179 |
| | MEEI | 73 |
| | BMC | 33 |
| | HVMA | 9 |
| Jackson Heart Study | | 606 |
| Total | | 890 |

*UMMC* University of Mississippi Medical Center, *MEEI* Massachusetts Eye and Ear Infirmary, *BMC* Boston Medical Center, *HVMA* Harvard Vanguard Medical Associates

cholesterol, LDL cholesterol, and triglycerides were evaluated as continuous variables for maximal power.

## Statistical analyses

We compared the covariates between cases and controls. Categorical variables were compared using the chi-square test, and continuous variables were compared with the *t* test. To examine the association between serum lipid levels and the presence of hard exudates, we used univariate and multivariate logistic regression. To examine the association between serum lipid levels and hard exudate area, we used linear regression for both the univariate and multivariate analyses. For all analyses, we used the subset of participants with complete information for the covariate of interest in that particular analysis to maximize the generalizability and power of the analysis. All multivariate regression models included age, gender, recruitment site, duration of diabetes, $HbA_{1c}$, mean systolic blood pressure, ETDRS diabetic retinopathy grade, presence of CSME, use of lipid-lowering medication, and use of anti-hypertensive medication. Since some have reported that laser photocoagulation can lead to increased lipid deposition [13], we also performed the analyses examining the association between serum lipid levels and hard exudates in the subgroup of patients who did not have focal laser scars on their fundus photography.

Because CSME is a visually important complication, we used univariate and multivariate linear regression to assess the association between presence of CSME and hard exudate area. The multivariate model included age, gender, recruitment site, duration of diabetes, $HbA_{1c}$, mean systolic blood pressure, and ETDRS diabetic retinopathy grade as covariates.

Inter-observer agreement for presence of CSME between the two graders was assessed with the kappa statistic. In the subgroup of patients with available CSMT from OCT imaging, agreement between OCT-determined and fundus photography-determined macular edema were evaluated with the Kappa statistic. Kappa was interpreted as follows: < 0 = less than chance agreement, 0.01–0.20 = slight agreement, 0.21–0.40 = fair agreement, 0.41–0.60 = moderate agreement, 0.61–0.80 = substantial agreement and 0.81–0.99 = almost perfect agreement [14]. Inter- and intra-observer reliability for HE area measurement was assessed with the interclass correlation coefficient (ICC). ICC was interpreted as follows: 0–0.2 = poor agreement, 0.3–0.4 = fair agreement, 0.5–0.6 = moderate agreement, 0.7–0.8 = strong agreement and >0.8 = almost perfect agreement [15]. The Bland–Altman method was also used to assess inter-observer agreement. In a Bland–Altman analysis, the agreement between two measurements is assessed by taking the difference between the two measurements and assessing the summary statistics and graphics of the differences [16–18]. The limits of agreement aim to describe how much the two measurements agree (or differ) using the estimated mean of the difference and the standard deviation of the differences. All analyses were performed using Stata/IC 12.1 (College Station, TX, USA). For the univariate and multivariate analyses, a $P$ value < 0.05 was considered statistically significant.

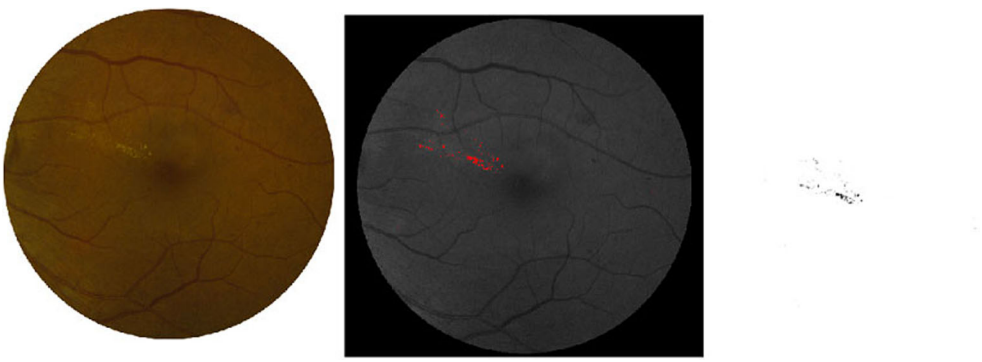

**Fig. 1** Quantitative assessment of the area covered by hard exudates. (*Left*) Color fundus photograph cropped to circle centered on the fovea with a radius equal to the distance between the fovea center and the temporal optic nerve margin. (*Middle*) The area covered by hard exudates was identified with the automatic thresholding function and manually (*red color area*). (*Right*) The total area covered by hard exudates was extracted and measured

## Results

890 patients (312 men and 578 women) met the inclusion criteria for this study. 169 patients (19 %) had hard exudates in at least one eye. Table 3 summarizes the clinical characteristics of patients with and without hard exudates. Participants with hard exudates had a longer duration of diabetes, higher mean $HbA_{1c}$ and higher mean systolic blood pressure. As expected, presence of CSME was associated with increased hard exudate area in the univariate analysis ($P = 3.0 \times 10^{-17}$) and also after adjusting for covariates in the multivariate analysis ($P = 2.1 \times 10^{-10}$).

Kappa agreement between the two graders for presence of CSME was 97.5 which represents a very high degree of agreement. Kappa agreement was 84.3 between OCT-based and fundus photography-based detection of macular edema which also reflects a high degree of agreement between the two methods. ICC for inter- and intra-observer agreement on total hard exudate area estimates were 0.999 for both, indicating the method to be highly reproducible. Bland–Altman agreement is shown in Fig. 2. The mean difference in hard exudate area between readers was 0.003 and the 95 % confidence interval (CI) was 0.012 to 0.019.

Table 4 shows the results of the univariate and multivariate analyses examining the primary endpoint: the association between serum lipid levels (per 10 mg/dl) and presence of hard exudates. In the univariate analysis, presence of hard exudates was associated with higher total cholesterol levels [odds ratio (OR) =1.05, 95 % CI 1.01–1.08, $P = 0.004$]. In the multivariate model, after controlling for other retinopathy risk factors, higher total cholesterol was still significantly associated with presence of exudates (OR = 1.08, 95 % CI 1.03–1.13, $P = 0.001$). Higher LDL was also associated with presence of hard exudates in the univariate analysis (OR = 1.05, 95 % CI 1.01–1.09, $P = 0.01$). This association was still significant after adjusting for other risk factors (OR = 1.08, 95 % CI 1.03–1.14, $P = 0.005$). Therefore, the risk of having hard exudates increases 8 % for every 10 mg/dl increase in total cholesterol and also for every 10 mg/dl increase in LDL cholesterol. Neither HDL cholesterol nor triglycerides were associated with presence of retinal hard exudates.

Table 5 shows the results of the secondary analysis: association between serum lipid levels and relative area of hard exudates in the 169 patients who had hard exudates. Univariate analyses showed statistically significant associations between higher total cholesterol levels and higher LDL levels and increased hard exudate area ($P = 0.02$ and $P = 0.003$, respectively). In the multivariate linear regression model adjusting for other covariates, these associations remained significant ($P = 0.04$ and $P = 0.01$, respectively). There were no significant associations between HDL cholesterol or triglycerides levels and relative hard exudate area. In the subset of 847 patients without focal laser scars on fundus photography, higher total and LDL cholesterol were still associated

**Table 3** Clinical characteristics of participants with and without hard exudates

| | Hard exudates present | | No hard exudates present | | |
|---|---|---|---|---|---|
| Variables | N | % or Mean ± SD | N | % or Mean ± SD | *P* value* |
| Age (years) | 169 | 62 ± 11.3 | 721 | 65 ± 10.6 | 0.0004 |
| Sex (male %) | 169 | 31.4 % | 721 | 36 % | 0.21 |
| Duration of diabetes (years) | 169 | 17.4 ± 10.3 | 721 | 11.5 ± 10.0 | $1.3 \times 10^{-10}$ |
| Hemoglobin A1C (%) | 169 | 8.4 ± 2.1 | 721 | 7.4 ± 1.7 | $1.3 \times 10^{-9}$ |
| Systolic blood pressure (mmHg) | 169 | 145.0 ± 26.8 | 721 | 133.5 ± 21.2 | $1.3 \times 10^{-8}$ |
| Lipid-lowering medication (%) | 91 | 54.1 % | 409 | 57.8 % | 0.54 |
| Anti-hypertensive medication (%) | 152 | 90.4 % | 633 | 88.4 % | 0.49 |
| Presence of clinically significant macular edema (%) | 108 | 64 % | 76 | 10.5 % | $2.5 \times 10^{-53}$ |
| Total cholesterol (mg/dl) | 169 | 195.0 ± 52.1 | 721 | 183.6 ± 44.4 | 0.004 |
| Low-density cholesterol (mg/dl) | 169 | 115.8 ± 45.9 | 721 | 107.1 ± 38.9 | 0.01 |
| High-density cholesterol (mg/dl) | 169 | 54.3 ± 18.3 | 721 | 53.4 ± 15.5 | 0.56 |
| Triglyceride (mg/dl) | 169 | 126.7 ± 73.2 | 721 | 117.3 ± 71.1 | 0.12 |
| ETDRS grade (% in each category) | 169 | | 721 | | $2.4 \times 10^{-51}$ |
| <15 | | 0 % | | 65 % | |
| 15–60 | | 42 % | | 16 % | |
| >60 | | 58 % | | 19 % | |

*SD* Standard deviation, *ETDRS* Early Treatment Diabetic Retinopathy Study

*Categorical variables compared using the chi-square test, and continuous variables compared with the *t* test

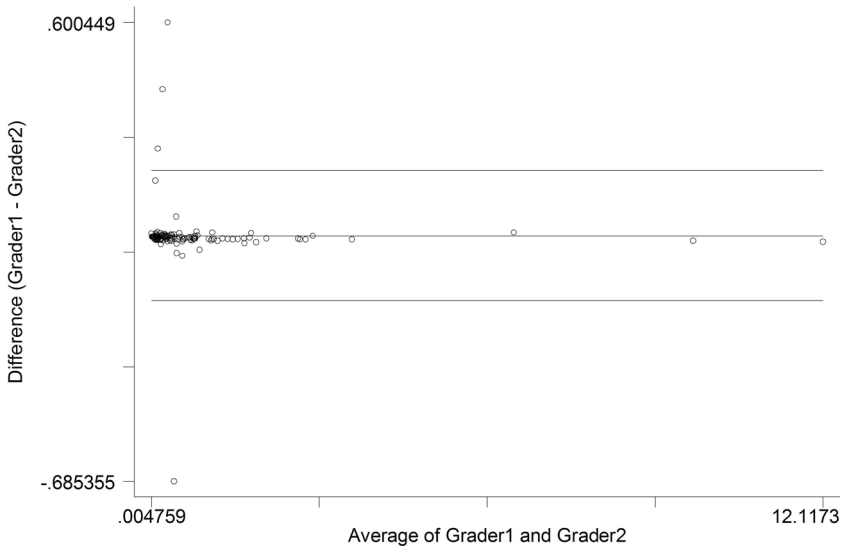


**Fig. 2** Bland–Altman agreement for hard exudate area between the two fundus photograph readers. The mean difference was 0.003 and 95 % limits of agreement were 0.005 to 12.117

with presence of hard exudates and greater hard exudate area to a statistically significant degree in multivariate analyses (all $P < 0.05$).

## Discussion

In this large sample of African Americans with T2D, greater hard exudate area and presence of hard exudates were associated with higher total and LDL cholesterol after adjusting for other diabetic retinopathy risk factors. The association is moderate in size. If a participant had a total cholesterol level that was 25 mg/dl lower compared to another participant, their risk of having hard exudates was 20 % lower. Other epidemiologic studies, including the Wisconsin Epidemiology Study of Diabetic Retinopathy (WESDR), have found similar associations between higher total or/and LDL cholesterol levels and hard exudate presence with qualitative assessment methods [5–7, 19–23]. LDL cholesterol has specifically been associated with presence and severity of hard exudates in African American patients with type 1 diabetes [5]. On the other hand, there are studies, including one that included some African American participants, which found other or no lipid fractions to be associated with macular exudates [6, 24, 25]. While our study is not definitive, it adds data from a significant number of patients to the literature and may help in future meta-analysis efforts that examine lipid levels and hard exudates.

One unique feature of this study is it examined not only presence vs. absence of exudates but also the area of hard exudate. Measurement of hard exudate area was highly reproducible between readers with the method we employed. To our best knowledge, there is only one other study that has examined hard exudate area and serum lipid levels [24]. This prior study was performed in 97 participants who were primarily English-speaking and had both T1D and T2D. As in our study, they found higher total and LDL cholesterol were associated with increased total hard exudate area in the univariate analyses. However, in their multivariate analyses, total cholesterol was no longer associated but LDL cholesterol and triglyceride levels were independently associated with

**Table 4** Association of serum lipid levels and presence of hard exudate

| | Univariate | | Multivariate* | |
|---|---|---|---|---|
| | OR (95 % CI) | *P* value | OR (95 % CI) | *P* value |
| Total cholesterol (per 10 mg/dl) | 1.05 (1.01–1.08) | 0.004 | 1.08 (1.03–1.13) | 0.001 |
| Low-density cholesterol (per 10 mg/dl) | 1.05 (1.01–1.09) | 0.01 | 1.08 (1.03–1.14) | 0.005 |
| High-density cholesterol (per 10 mg/dl) | 1.03 (0.92–1.14) | 0.58 | 1.06 (0.93–1.22) | 0.351 |
| Triglyceride (per 10 mg/dl) | 1.01 (0.99–1.03) | 0.13 | 1.02 (0.99–1.05) | 0.126 |

*OR* odds ratio, *CI* confidence interval

* adjusted for age, gender, diabetes duration, hemoglobin A1C, systolic blood pressure, lipid-lowering medication, anti-hypertensive medication, Early Treatment Diabetic Retinopathy Study grade, presence of clinically significant macular edema and site

**Table 5** Linear regression results for the relationship between serum lipid levels and retinal hard exudate area

| | Univariate | | Multivariate* | |
|---|---|---|---|---|
| | Coef (95 % CI) | *P* value | Coef (95 % CI) | *P* value |
| Total cholesterol (mg/dl) | 0.001 (0.0002–0.0019) | 0.020 | 0.001 (0.00004–0.0020) | 0.040 |
| Low-density cholesterol (mg/dl) | 0.001 (0.0004–0.002) | 0.003 | 0.001 (0.0002–0.002) | 0.018 |
| High-density cholesterol (mg/dl) | 0.001 (−0.0009–0.004) | 0.161 | 0.0020 (−0.0006–0.0050) | 0.145 |
| Triglyceride (mg/dl) | 0.0002 (−0.0003–0.0008) | 0.501 | 0.0001 (−0.0005–0.0007) | 0.718 |

*Coef* beta coefficient from linear regression per mg/dl change in lipid level, *CI* confidence interval

* adjusted for age, gender, diabetes duration, hemoglobin A1C, systolic blood pressure, lipid-lowering medication, anti-hypertensive medication, Early Treatment Diabetic Retinopathy Study grade, presence of clinically significant macular edema and site

increased total hard exudate area. The difference in results between the two studies may be due to the different ethnic populations examined, the inclusion of participants with type 1 diabetes in the prior study, and differences in hard exudate area measurement techniques.

Several factors need to be taken into account when measuring hard exudate area. With the currently available software with commercial fundus cameras, it is not possible to precisely measure true lesion size on digital fundus images directly. A distance can be measured in pixels but it cannot be converted into an actual size in millimeters. This is because of variability in image magnification produced by differences among patients with regards to corneal curvature, axial length, and refractive error [26]. Some investigators have tried to use the optic disc diameter as a landmark [24] by which to calibrate magnification, but this carries an inherent error as horizontal disc diameters range from 0.91 mm to 2.42 mm (mean 1.79 ± 0.27 mm) [27–29]. Calibration of retinal images based on fovea–nerve distance is more precise than optic disc diameter but this distance also varies significantly between 3.91 and 4.90 mm (mean 4.32 ± 0.32 mm) [24, 26]. Thus, we chose to use relative hard exudate area within a predetermined circle around the fovea because we felt it was the most accurate method and circumvented the inaccuracies from variability in magnification due to the various factors listed above. However, our method also has limitations. It only allows calculation of relative area, not an absolute area. We also evaluated a small amount of the retina outside the arcades, e.g. beyond the classically described macular region.

The associations we found between lipid levels and hard exudate area are consistent with data from clinical trials [30–32]. Oral atorvastatin therapy in patients with T2D and dyslipidemia reduces the severity of hard exudates and subfoveal lipid migration in CSME [31]. The Action to Control Cardiovascular Risk in Diabetes Study confirmed the benefits of fenofibrate, a lipid-lowering agent, in reducing diabetic retinopathy progression although they did not specifically look at macular hard exudate. Participants in this study were from multiple ethnicities: white, African American, Hispanic and others [30].Unfortunately, these trials cannot identify which changes in specific lipid fractions are responsible for the clinical effects observed.

One strength of this study is that it was performed in a single ethnic group, African Americans, and in a single diabetes subtype, T2D thus limiting the heterogeneity in the clinical sample. There are substantial data that demonstrate variation in overall diabetic retinopathy prevalence and macular edema by race/ethnicity. Three studies, the Atherosclerosis Risk in Communities Study,[6] the National Health and Nutrition Examination Survey (NHANES) III [33], and the Cardiovascular Health Study [34], showed that retinopathy is more prevalent in blacks with T2D than in whites. The Multi-ethnic Study of Atherosclerosis showed that the prevalence of any diabetic retinopathy and macular edema was significantly higher in blacks and Hispanics than in whites and Chinese [35]. A cross-sectional analysis of NHANES showed a greater burden of DME among non-Hispanic blacks [36]. No study has specifically compared African Americans to other ethnicities with regards to hard exudates. Hard exudate deposition may also vary according to diabetes type. The WESDR showed there were different results for the association between hard exudates and lipid serum levels according to whether the participants had non-insulin- vs. insulin-dependent diabetes mellitus [19].

There are some limitations to our study that should be considered. A reading center was not used for photograph grading, but readers were ophthalmologists and quality metrics indicated high inter-grader agreement and no significant temporal drift in severity grading [9]. The cross-sectional design of our study does not allow us to judge the causal or temporal relationships of the associations we found. Our samples were clinic- and community-based rather than population-based and this limits the generalizability of the results to the overall African American population. Statins have been proven to be effective for primary and secondary prevention of cardiovascular accidents in diabetes patients

[37]. Because statins are so widely prescribed for diabetic patients, the great majority of our patients were on a statin and a small number taking a statin plus fenofibrate. Therefore, we did not have sufficient power to examine the effects of different lipid-lowering medications on hard exudate area. Finally, it has been shown that some DME treatment methods can influence the amount of hard exudate. For example, some have reported that laser photocoagulation can lead to increased lipid deposition [13]. Unfortunately, similar to the previous study on this topic [24], we could not include previous DME treatment method as a covariate in our analyses because we did not have sufficiently detailed information regarding treatment for DME on most of our patients. However, we did a subgroup analysis excluding patients with focal laser scars on fundus photography, and the results were still significant with higher total and LDL cholesterol levels being associated with presence of hard exudates and greater hard exudate area.

In summary, we found associations between both higher total cholesterol and LDL and presence of hard exudate and hard exudate area in African Americans with type 2 diabetes. This incrementally adds support to the existing evidence that serum lipid levels influence the formation and extent of hard exudate deposition in the retina. This data can be used in counseling patients with diabetes about the importance of controlling serum lipid levels for decreasing their risk of developing potentially visually disabling macular hard exudates.

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### Compliance with ethical standards

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**Conflict of interest** All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript. None of the authors have any proprietary interests or conflicts of interest related to this submission.

**Ethical approval** All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki Declaration and its later amendments.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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