Serum Apolipoprotein AI and B Are Stronger Biomarkers of Diabetic Retinopathy Than Traditional Lipids

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OBJECTIVE—To describe and compare the associations of serum lipoproteins and apolipoproteins with diabetic retinopathy.

RESEARCH DESIGN AND METHODS—This was a cross-sectional study of 224 diabetic patients (85 type 1 and 139 type 2) from a diabetes clinic. Diabetic retinopathy was graded from fundus photographs according to the Airlie House Classification system and categorized into mild, moderate, and vision-threatening diabetic retinopathy (VTDR). Serum traditional lipids (total, LDL, non–HDL, and HDL cholesterol and triglycerides) and apolipoprotein AI (apoAI), apolipoprotein B (apoB), and the apoB-to-apoAI ratio were assessed.

RESULTS—Diabetic retinopathy was present in 133 (59.4%) individuals. After adjustment for age, sex, diabetes duration, A1C, systolic blood pressure, and diabetes medications, the HDL cholesterol level was inversely associated with diabetic retinopathy (odds ratio 0.39 [95% CI 0.16–0.94], highest versus lowest quartile; P_{trend} = 0.017). The ApoAI level was inversely associated with diabetic retinopathy (per SD increase, 0.76 [95% CI 0.59–0.98]), whereas apoB (per SD increase, 1.31 [1.02–1.68]) and the apoB-to-apoAI ratio (per SD increase, 1.48 [1.13–1.95]) were positively associated with diabetic retinopathy. Results were similar for mild to moderate diabetic retinopathy and VTDR. Traditional lipid levels improved the area under the receiver operating curve by 1.8%, whereas apolipoproteins improved the area by 8.2%.

CONCLUSIONS—ApoAI and apoB and the apoB-to-apoAI ratio were significantly and independently associated with diabetic retinopathy and diabetic retinopathy severity and improved the ability to discriminate diabetic retinopathy by 8%. Serum apolipoprotein levels may therefore be stronger biomarkers of diabetic retinopathy than traditional lipid measures.

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Diabetic retinopathy remains the leading cause of morbidity and disability in people with diabetes (1). Whereas diabetes duration, hyperglycemia, and hypertension are established diabetic retinopathy risk factors, the current understanding of other risk factors for diabetic retinopathy remains poor (2,3).

There is controversy regarding the role of lipids in the pathogenesis of diabetic retinopathy (4–6). Data from the Diabetes Control and Complications Trial (DCCT) showed that traditional measures of serum lipids (e.g., triglycerides) were positively associated with the risk of diabetic retinopathy in type 1 diabetes (6). However, other studies have not consistently shown similar associations (4,7). Recent data from the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) Study indicated that fenofibrate, a lipid-altering medication, reduced diabetic retinopathy progression and the need for laser treatment in type 2 diabetes (7). This benefit was unrelated to serum lipid levels, with unclear underlying mechanisms.

Recently, there has been an interest in the relationship of apolipoprotein AI (apoAI) and apolipoprotein B (apoB) with diabetic retinopathy (5,8,9). ApoAI is an HDL constituent and apoB is present in VLDL, intermediate-density lipoprotein (IDL), LDL, and lipoprotein(a), and both apolipoproteins are not affected by prandial status (10). Because apoAI better reflects lipid accumulation in peripheral tissues (8) and apoB is present in the retina of human eyes with diabetic retinopathy (11), they may be more directly relevant to the biophysiological changes associated with diabetic retinopathy than the traditional lipids (e.g., HDL and LDL cholesterol and triglycerides) (10). However, the extent to which apolipoprotein measures are useful to identify individuals at risk of diabetic retinopathy remains unknown. In this study we aimed to evaluate and compare the associations of apolipoproteins and traditional lipid profiles with diabetic retinopathy in adults with diabetes.

RESEARCH DESIGN AND METHODS

—This was a clinic-based observational study. We consecutively recruited 224 Caucasians with diabetes aged 18–70 years (85 with type 1 diabetes and 139 with type 2 diabetes) between October 2006 and April 2008 from the eye clinics at the International Diabetes Institute, Melbourne, Victoria, Australia (12). Participants were excluded if they had a history of epilepsy or glaucoma, had undergone previous vitreal surgery, and/or had a cataract on examination. The study followed the tenets of the Declaration of Helsinki and was approved by the Institute ethics committee, with written informed consent obtained from each participant.

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Assessment of diabetic retinopathy

Participants had a standardized clinical examination and retinal photography. Diabetic retinopathy was graded from the digital retinal photographs at the Retinal Vascular Imaging Centre, Centre for Eye Research Australia by graders masked to participants' clinical details. A diabetic retinopathy severity score was assigned for each eye according to the modified Airlie House Classification system (13). We defined "no diabetic retinopathy" as levels 10–12, mild nonproliferative diabetic retinopathy (NPDR) as levels 14–20, moderate NPDR as levels 31 and 41, and severe NPDR and proliferative diabetic retinopathy (PDR) as levels 51–80. Any diabetic retinopathy was defined as levels 14–80. Macular edema was defined as present or absent and classified as with or without clinically significant macular edema (CSME). Vision-threatening diabetic retinopathy (VTDR) was defined to include severe NPDR, PDR, and CSME.

Blood chemistry

Fasting (>8 h) blood samples were drawn from participants at local pathology centers to assess fasting blood glucose level, serum lipids (total, HDL, and LDL cholesterol and triglycerides) and apolipoprotein (apoAI and apoB) levels, and A1C within 2 weeks of eye examinations. Non–HDL cholesterol was calculated by total cholesterol minus HDL cholesterol. LDL cholesterol was measured using an LDL cholesterol direct assay. In addition, on the day of eye examinations, venous blood samples were drawn from each participant, centrifuged (Heraeus-Labofuge 400R, 1000*g*, 10 min, 4°C), and then aliquoted into polypropylene tubes. All aliquots were initially stored (−20°C) at the International Diabetes Institute and subsequently (within 2 weeks) were transferred on dry ice to storage at −80°C. Serum apoAI and apoB were measured later on these samples using rate immunonephelometry (Dade Behring BN II Nephelometer; Siemens Healthcare Diagnostics, Eschborn, Germany) with kits from the same company at the Department of Medicine, the University of Melbourne, St. Vincent's Hospital (Melbourne, VIC, Australia). Intra-assay coefficients of variation for apoAI and apoB were 2.2 and 1.9%, respectively, and inter-assay coefficients of variation were 5.7 and 2.4%, respectively.

Assessment of other risk factors

All participants underwent a standardized clinical examination and interview using a detailed questionnaire to obtain information including past medical history, current cigarette smoking status, and the use of antihypertensive medications, lipid-lowering medications, and oral hypoglycemic agents. Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg, or current use of antihypertensive medications. Height and weight were measured to determine BMI.

Statistical analysis

Analyses were performed using Intercooled Stata (version 10.1 for Windows; StataCorp, College Station, TX). Baseline characteristics of participants with and without diabetic retinopathy were compared using a χ^2 test for proportions, *t* test, or Mann-Whitney *U* test for means. Serum lipid and apolipoprotein variables were assessed categorically (in quartiles) and continuously (per SD change). An individual's diabetic retinopathy level was based on the diabetic retinopathy level of the worse eye. Logistic regression was used to assess the association between serum lipids and apolipoproteins and diabetic retinopathy. Multinomial and ordered logistic regression models were performed to assess associations between serum lipids or apolipoproteins and diabetic retinopathy severity categories (mild and moderate NPDR and VTDR). We initially adjusted for age and sex (model 1) and in addition for diabetes duration, A1C, SBP, BMI, use of diabetes medications, use of lipid-lowering medications, and insulin use (model 2). Covariables included in the models were either continuous (per SD changes for age, SBP, and BMI; per year for duration; and per percentage for A1C) or categorical. We constructed models for diabetic retinopathy prevalence containing either traditional lipids or apolipoproteins in the models and used the receiver operator characteristic (ROC) curve to compare the discrimination ability of these models. Area under the ROC curve (AUC) and the percentage of the incremental changes in AUC to models with age, sex, duration of diabetes, A1C, and SBP were assessed.

RESULTS—Of the 224 diabetic study participants, 13.8% (31) had mild NPDR, 22.3% (50) had moderate NPDR, and 23.3% (52) had VTDR. Participants with diabetic retinopathy had longer diabetes duration, higher A1C, and higher SBP levels than those without diabetic retinopathy (Table 1). Mean levels of traditional lipids were not significantly different between participants with and

Table 1—*Baseline characteristics of 224 participants with diabetes, according to retinopathy status*

Characteristics	Retinopathy status		*P* value*
	No	Yes	
n	95	129	
Male sex	55.8	62.0	0.35
Current cigarette smoker	58.1	50.4	0.11
Use of insulin	57.9	71.3	0.037
Use of oral hypoglycemic agents	53.7	55.8	0.75
Use of lipid-lowering medication	46.3	58.1	0.080
Age (years)	58 (49, 66)	60 (52, 66)	0.43
BMI (kg/m^2)	29.5 ± 5.6	31.1 ± 6.6	0.070
SBP (mmHg)	125.2 ± 13.1	130.1 ± 15.1	0.011
Duration of diabetes (years)	10 (6, 17)	18 (10, 24)	<0.001
A1C (%)	7.6 ± 1.7	8.0 ± 1.2	0.022
Cholesterol (mmol/L)	4.7 ± 1.1	4.5 ± 1.1	0.47
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.3 ± 0.5	0.056
Non–HDL cholesterol (mmol/L)	3.3 ± 1.0	3.2 ± 1.1	0.85
LDL cholesterol (mmol/L)	2.5 ± 0.7	2.5 ± 0.9	0.99
Triglyceride (mmol/L)	1.3 (0.9, 1.9)	1.3 (0.9, 1.9)	0.50
ApoAI (g/L)	1.5 ± 0.2	1.4 ± 0.3	0.001
ApoB (g/L)	0.8 ± 0.2	0.9 ± 0.3	0.015
ApoB-to-apoAI ratio	0.6 ± 0.2	0.7 ± 0.2	<0.001

Data are %, mean ± SD, or median (25th, 75th percentile). **P* values were obtained using a χ^2 test (categorical), a *t* test (continuous and normally distributed), or the Mann-Whitney *U* test (continuous and skewed), comparing diabetic participants with and without retinopathy.

without diabetic retinopathy. The serum apoAI level was lower, and both serum apoB and the apoB-to-apoAI ratio were higher in participants with diabetic retinopathy than in those without diabetic retinopathy ($P = 0.001$, $P = 0.015$, and $P < 0.001$ consecutively) (Table 1).

After adjustment for age and sex, longer diabetes duration, higher A1C, and higher SBP levels were associated with increasing severity of diabetic retinopathy ($P_{trend} < 0.0001$, 0.004, and 0.011, respectively) (Supplementary Table A1). Table 2 shows associations between serum lipids, apolipoproteins, and diabetic retinopathy. After adjustment for all covariables in model 2, HDL cholesterol levels were inversely associated with any diabetic retinopathy (odds ratio [OR] 0.39 [95% CI 0.16–0.94]; comparing the highest with the lowest quartile, $P_{trend} = 0.017$). Per SD increase in apoAI (0.76 [0.59–0.98]), apoB (1.31 [1.02–1.68]), or the ratio of apoB-to-apoAI ratio (1.48 [1.13–1.95]) was strongly associated with diabetic retinopathy. Additional adjustment for HDL cholesterol levels did not alter the associations of apolipoproteins with diabetic retinopathy.

Table 3 shows associations of serum lipids or apolipoproteins with diabetic retinopathy severity. After adjustment for all covariables (model 2), per SD increase in apoAI was associated with a reduced likelihood of having more severe diabetic retinopathy levels ($P_{trend} = 0.001$). Likewise, increasing levels of apoB ($P_{trend} = 0.02$) and the apoB-to-apoAI ratio ($P_{trend} = 0.001$) were associated with increasing diabetic retinopathy severity levels. In supplementary analyses stratifying VTDR into severe NPDR ($n = 17$), PDR ($n = 28$), and CSME ($n = 7$), the association between apoAI and diabetic retinopathy severity remained significant ($P_{trend} = 0.02$). However, the association between apoB ($P_{trend} = 0.06$) or the apoB-to-apoAI ratio ($P_{trend} = 0.05$) and diabetic retinopathy severity became marginally nonsignificant (Supplementary Table A2). Further stratification by sex and diabetes type showed that findings were largely similar in men and women (Supplementary Tables A3 and A5) and in type 1 and type 2 diabetes (Supplementary Tables A4, A6, and A7), except for the association between HDL cholesterol and diabetic retinopathy.

Table 4 shows the AUC for predicting diabetic retinopathy with traditional lipids or apolipoproteins in addition to the established diabetic retinopathy risk

Table 2—*Associations of serum lipids with any diabetic retinopathy*

Serum lipids	% of events	Any retinopathy			
		Model 1*	*P* value	Model 2†	*P* value
Cholesterol (mmol/L)					
1st quartile, <3.8	16.4	1.00		1.00	
2nd quartile, 3.8-4.5	12.8	0.91 (0.53–1.57)		0.73 (0.40–1.35)	
3rd quartile, 4.5-5.2	14.6	1.02 (0.59–1.76)		0.88 (0.47–1.66)	
4th quartile, ≥5.2	14.2	1.03 (0.60–1.78)	0.82*	0.90 (0.47–1.74)	0.89*
Per SD increase (1.1)		0.94 (0.78–1.14)	0.54	0.91 (0.72–1.14)	0.40
HDL cholesterol (mmol/L)					
1st quartile, <0.9	21.2	1.00		1.00	
2nd quartile, 0.9-1.3	16.4	0.84 (0.40–1.75)		0.80 (0.34–1.89)	
3rd quartile, 1.3-1.9	13.1	0.46 (0.22–0.95)		0.37 (0.15–0.86)	
4th quartile, ≥1.9	12.0	0.39 (0.19–0.79)	0.003*	0.39 (0.16–0.94)	0.017*
Per SD decrease (0.5)		0.84 (0.66–1.08)	0.182	0.94 (0.71–1.24)	0.44
Non–HDL cholesterol (mmol/L)					
1st quartile, <1.9	16.4	1.00		1.00	
2nd quartile, 1.9-2.4	13.1	0.68 (0.34–1.35)		0.65 (0.30–1.42)	
3rd quartile, 2.4-2.9	16.1	1.13 (0.55–2.32)		0.93 (0.42–2.06)	
4th quartile, ≥2.9	15.0	0.95 (0.47–1.92)	0.75*	0.70 (0.29–1.65)	0.61*
Per SD increase (0.8)		1.00 (0.79–1.29)	0.96	0.87 (0.65–1.18)	0.38
LDL cholesterol (mmol/L)					
1st quartile, <1.9	14.9	1.00		1.00	
2nd quartile, 1.9-2.4	18.2	1.56 (0.77–3.16)		1.20 (0.52–2.76)	
3rd quartile, 2.4-2.9	10.9	0.71 (0.34–1.46)		0.43 (0.18–1.06)	
4th quartile, ≥2.9	14.9	1.31 (0.63–2.71)	0.95*	1.07 (0.45–2.53)	0.67*
Per SD increase (0.8)		1.12 (0.86–1.46)	0.40	1.07 (0.77–1.48)	0.68
Triglyceride (mmol/L)					
1st quartile, <0.9	18.5	1.00		1.00	
2nd quartile, 0.9-1.3	13.1	1.00 (0.59–1.72)		0.91 (0.50–1.66)	
3rd quartile, 1.3-1.9	12.2	0.77 (0.45–1.31)		0.73 (0.38–1.41)	
4th quartile, ≥1.9	14.4	1.03 (0.60–1.76)	0.82*	0.90 (0.47–1.73)	0.65*
Per SD increase (1.1)		0.98 (0.81–1.20)	0.88	0.95 (0.75–1.19)	0.65
ApoAI (g/L)					
1st quartile, <0.9	19.2	1.00		1.00	
2nd quartile, 0.9-1.3	13.9	0.45 (0.25–0.82)		0.37 (0.19–0.72)	
3rd quartile, 1.3-1.9	13.7	0.39 (0.22–0.69)		0.48 (0.24–0.96)	
4th quartile, ≥1.9	10.6	0.26 (0.15–0.48)	<0.001*	0.33 (0.16–0.69)	0.015*
Per SD decrease (0.3)		0.67 (0.54–0.83)	<0.001	0.76 (0.59–0.98)	0.034
ApoB (g/L)					
1st quartile, <0.9	11.3	1.00		1.00	
2nd quartile, 0.9-1.3	15.0	1.90 (1.10–3.27)		2.10 (1.15–3.82)	
3rd quartile, 1.3-1.9	15.0	1.92 (1.14–3.31)		1.89 (1.03–3.43)	
4th quartile, ≥1.9	16.4	2.69 (1.53–4.73)	0.001*	2.69 (1.39–5.20)	0.005*
Per SD increase (0.3)		1.36 (1.10–1.54)	0.004	1.31 (1.02–1.68)	0.035
ApoB-to-apoAI ratio					
1st quartile, <0.9	11.8	1.00		1.00	
2nd quartile, 0.9-1.3	12.7	1.23 (0.72–2.09)		0.99 (0.55–1.80)	
3rd quartile, 1.3-1.9	15.3	1.86 (1.08–3.20)		1.62 (0.88–2.97)	
4th quartile, ≥ 1.9	17.6	2.84 (1.61–5.00)	<0.001*	2.13 (1.07–4.23)	0.017*
Per SD increase (0.2)		1.60 (1.28–2.00)	<0.001	1.48 (1.13–1.95)	0.005

Data are ORs (95% CI). Each risk factor is in separate models. Model 1: adjusted for age and sex. Model 2: model 1 plus adjusted for duration of diabetes, A1C, SBP, BMI, use of diabetic medications, lipid-lowering agents, and insulin. Age, duration of diabetes, A1C, SBP, and BMI were treated as continuous variables. *P_{trend}.

Table 3—*Associations of serum lipids with severity of diabetic retinopathy*

Serum lipids (per SD increase)	Mild DR	Moderate DR	VTDR	P_{trend}
n	31	50	52	
Model 1				
Cholesterol (1.1) (mmol/L)	1.00 (0.74–1.34)	0.93 (0.72–1.20)	0.93 (0.72–1.19)	0.47
Triglyceride (1.1) (mmol/L)	0.94 (0.68–1.29)	0.88 (0.67–1.16)	1.10 (0.87–1.39)	0.72
HDL cholesterol (0.5) (mmol/L)	0.85 (0.56–1.31)	0.93 (0.68–1.27)	0.77 (0.55–1.06)	0.15
LDL cholesterol (0.8) (mmol/L)	1.00 (0.64–1.57)	1.08 (0.78–1.50)	1.20 (0.88–1.64)	0.26
ApoAI (0.3) (g/L)	0.87 (0.62–1.20)	0.78 (0.59–1.02)	0.48 (0.35–0.64)	<0.001
ApoB (0.3) (g/L)	1.31 (0.95–1.79)	1.33 (1.03–1.72)	1.44 (1.11–1.85)	0.002
ApoB-to-apoAI ratio (0.2)	1.43 (1.03–1.99)	1.45 (1.10–1.90)	1.90 (1.46–2.48)	<0.001
Model 2				
Cholesterol (1.1) (mmol/L)	0.97 (0.69–1.36)	0.87 (0.65–1.17)	0.90 (0.66–1.22)	0.19
Triglyceride (1.1) (mmol/L)	0.92 (0.64–1.33)	0.83 (0.61–1.15)	1.06 (0.80–1.40)	0.95
HDL cholesterol (0.5) (mmol/L)	0.90 (0.52–1.54)	0.97 (0.64–1.48)	0.81 (1.54–1.21)	0.30
LDL cholesterol (0.8) (mmol/L)	0.95 (0.58–1.54)	1.04 (0.70–1.53)	1.19 (0.80–1.77)	0.73
ApoAI (0.3) (g/L)	0.86 (0.59–1.27)	0.93 (0.68–1.29)	0.53 (0.38–0.76)	0.001
ApoB (0.3) (g/L)	1.40 (1.00–1.97)	1.27 (0.95–1.71)	1.47 (1.10–1.96)	0.020
ApoB-to-apoAI ratio (0.2)	1.58 (1.07–2.32)	1.22 (0.87–1.69)	1.76 (1.27–2.45)	0.001

Data are ORs (95% CI). Each risk factor is in separate models, calculated per SD increase of serum lipids. P_{trend} was calculated using ordered logistic regression. Model 1: adjusted for age and sex. Model 2: model 1 plus adjusted for duration of diabetes, A1C, SBP, BMI, use of diabetic medications, lipid-lowering agents, and insulin. Age, duration of diabetes, A1C, SBP, and BMI were treated as continuous variables. DR, diabetic retinopathy.

factors including age, sex, diabetes duration, A1C, and SBP. Total percent change of the AUC by addition of traditional lipids into the basic model was 1.8%, whereas addition of apolipoproteins improved the AUC by up to 8.2%.

CONCLUSIONS—Serum lipids have long been proposed to be risk factors for diabetic retinopathy (4,14), although the relationship of lipids to diabetic retinopathy has been relatively understudied compared with diabetes duration, A1C, and blood pressure. Our study demonstrated that of the traditional lipid measures, only lower HDL cholesterol was independently associated with diabetic retinopathy. However, serum apoAI, apoB, and the apoB-to-apoAI ratio were consistently associated with the presence of diabetic retinopathy and severity of diabetic retinopathy, independent of age, sex, and known diabetic retinopathy risk factors. Our findings support previous evidence that traditional serum lipids are not strongly or consistently associated with diabetic retinopathy.

Lipid associations with diabetic retinopathy have been investigated in multiple population-based studies and clinical trials, but findings remain inconsistent (4–6,13,15–17), with no single lipid measure consistently found to be associated with diabetic retinopathy. For example, total cholesterol was an independent risk factor for diabetic retinopathy in the Chennai Urban Rural Epidemiology Study (CURES) but was protective against diabetic retinopathy in the Singapore Malay Eye Study population (16,17). Similar to our findings, data from the DCCT did not show an association with total cholesterol levels but showed that the HDL cholesterol level was inversely associated with diabetic retinopathy in type 1 diabetes (5). Other studies reported that serum lipids were associated with retinal hard exudates and diabetic macular edema only (4,6,15). In contrast, the Multi-Ethnic Study of Atherosclerosis (MESA) showed no associations of serum lipids with diabetic retinopathy (13). A large randomized controlled trial in type 2 diabetes, the FIELD Study, showed that fenofibrate use over 5 years reduced the need for laser treatment for diabetic retinopathy and macular edema, although it did not affect diabetic retinopathy incidence (7). Because the effect of fenofibrate on diabetic retinopathy was not related to lipid level changes, its mechanism is not yet clear (18).

Very few reports from the literature are available to compare with our findings regarding apolipoproteins and diabetic retinopathy. Cross-sectional data from the DCCT/Epidemiology of Diabetes Interventions and Complications in type 1 diabetes demonstrated associations between diabetic retinopathy and serum lipoprotein subclass profiles (5) and between apoB, small LDL, and LDL particle concentration and diabetic retinopathy, suggesting the role of lipoprotein subclasses in the pathogenesis of diabetic retinopathy (5). Although the exact underlying mechanism is uncertain, we have strong evidence for the plausibility of our findings. HDL is the main vasoprotective lipoprotein (19). ApoAI, the main HDL structural protein that is produced by the liver and intestine, is essential for reverse transport of cholesterol from peripheral tissue to the liver. It also has antioxidant and anti-inflammatory effects (19). On the contrary, apoB is a major structural protein for VLDL, IDL, and LDL and is responsible for delivering lipids from the liver and intestine to peripheral tissue. Total apoB levels may reflect atherogenic potential (20). There are a few studies showing the presence of apoAI and apoB in intraocular specimens (8,9,11). Higher apoAI levels in vitreous fluid and retinal pigment epithelium among individuals with diabetes than in those without diabetes (8,9) suggest protective mechanisms within the retina via apoAI against lipid deposition and inflammation-induced lipotoxicity leading to diabetic retinopathy (8). Because apoAI has anti-inflammatory and anti-oxidant effects (19) and is also key to intraretinal lipid transport (21), it is conceivable that a low level of this protective agent may promote diabetic retinopathy. Furthermore, evidence showing increased levels of retinal apoB associated with greater severity of diabetic retinopathy suggests that higher apoB levels, which may reflect higher lipoprotein-related

Table 4—*Prediction model of traditional lipids and apolipoproteins for diabetic retinopathy using AUC*

	ROC curve for diabetic retinopathy	
Variables	AUC	% change in AUC*
Model 1: age, sex, duration of diabetes, A1C, SBP	0.690	
Model 2†		
Cholesterol	0.690	0.0
HDL cholesterol	0.701	1.8
Non–HDL cholesterol	0.684	−0.9
LDL cholesterol	0.686	−0.6
Triglyceride	0.692	0.3
Traditional lipids‡	0.701	1.8
Model 2†		
ApoAI	0.730	6.6
ApoB	0.723	5.4
ApoB-to-apoAI ratio	0.728	6.2
Apolipoproteins‡	0.740	8.2
Traditional lipids and apolipoproteins§	0.735	7.4

*% increase in AUC = (AUC model 2 − AUC model 1)/(AUC model 1) ×100. †Each variable was added separately to model 1. The AUC of each row is that of the variable and model 1 only. ‡The combined change of the AUC for the traditional lipids (total, HDL, non–HDL, and LDL cholesterol and triglyceride) or apolipoproteins (apoAI, apoB, and apoB-to-apoAI ratio). §The combined change of the AUC for the traditional lipids and apolipoproteins.

toxins, are destructive to arterial and retinal vascular cells (11).

Although apoAI and HDL or apoB and LDL are related, HDL and LDL do not show similar associations with diabetic retinopathy as apoAI and apoB do. There are a few reasons to explain why apoAI and apoB measurements are superior to HDL and LDL in individuals with diabetes (5,22). First, these apolipoproteins and HDL cholesterol and LDL cholesterol reflect different aspects of lipoprotein composition: apolipoproteins are protein moieties with structural, enzymatic, and receptor-binding functions, and HDL cholesterol and LDL cholesterol refer to the cholesterol lipid content of HDL and LDL only (10,20). Apolipoproteins occur in more than one lipoprotein class: apoAI is found predominantly in HDL, and there are several apoAI particles per HDL particle, whereas there is only one apoB moiety per particle of chylomicrons, VLDL, IDL, LDL, and lipoprotein(a) (10,20). The use of only HDL and LDL ignores the significant contribution from other lipoproteins, and, therefore, it is not surprising that HDL cholesterol and LDL cholesterol were not strongly correlated with diabetic retinopathy. Second, the diabetic milieu induces changes such as nonenzymatic glycation, oxidation, and advanced glycation end product modification of lipoproteins which may affect the assay results (23). Similarly, there may be differences in the precision of measurement of lipoproteins and potential for differential effects of sample handling and nonenzymatic glycation and oxidation on the different assays. On the contrary, apoAI and apoB levels are more stable than lipid levels, particularly in individuals with diabetes, and are not affected significantly by prandial status (5,10).

Our study may have clinical implications. Although apolipoprotein measurements have not been widely used in clinical practice, these measures seem to have distinct and more obvious associations with diabetic retinopathy than the traditional lipids. ApoAI, apoB, and the apoB-to-apoAI ratio cover both damaging and protective lipoprotein pathways. We showed that serum apoAI, apoB and the apoB-to-apoAI ratio have better ability to discriminate the presence and absence of diabetic retinopathy than traditional lipids, with an improvement in AUC to 8% compared with less than 2% from traditional lipids. These findings seem to support the concept that the regulatory mechanism of lipid deposition to and from target tissues (i.e., functions of apoB and apoAI), particularly the retina, may be more important than the concentration of circulating cholesterol in the pathogenesis of diabetic retinopathy (8,9,11,24).

The strengths of our study include the assessment of diabetic retinopathy by standardized grading protocols, and measurement of both traditional lipid and apolipoprotein levels. Despite the small sample size, our study sample was typical of that of other diabetic populations with diabetic retinopathy, showing strong associations with A1C level and diabetes duration. In addition, AUC for established risk factors for diabetic retinopathy is comparable between MESA (25) and our present study. We therefore believe, with good reasons, the generalizability of our findings to other diabetic populations. Limitations are noted. First, the cross-sectional design of this study does not provide information as to whether apoAI and apoB predict progression of diabetic retinopathy. Prospective studies are needed to assess the temporal sequence of these associations. Second, our diabetic retinopathy grading was based on only two (optic disc and macular) fields rather than on seven fields of retinal photographs used by large trials such as the Early Treatment Diabetic Retinopathy Study (ETDRS). We could have missed individuals with mild levels of diabetic retinopathy and underestimated diabetic retinopathy prevalence in our study sample. However, misclassification between case and control subjects will only bias the associations toward the null. If seven-field retinal photography were performed in our study, the observed associations would still have been present, if not stronger. Because of the relatively small sample size, no distinction was made between type 1 and type 2 diabetes in the primary analysis. However, we have further explored these associations in subgroups stratified by diabetes type. These associations remained in both subgroups of type 1 and type 2 diabetes (Supplementary Tables A4 and A6), although it seemed that the associations of apoB and the apoB-to-apoAI ratio with diabetic retinopathy were stronger in type 1 than in type 2 diabetes (Supplementary Table A6). Third, the number of participants with CSME in this study was very small (<5%); thus, we were unable to perform a specific analysis on CSME alone. Finally, the diabetic retinopathy prediction models and estimates were derived from and validated in the same study sample. Confirmation in other studies is needed.

In summary, we report new associations among serum apolipoproteins (apoAI, apoB, and the apoB-to-apoAI ratio) and the presence and severity of diabetic retinopathy in people with diabetes. Concurrently, we showed that apart from HDL cholesterol, conventional serum lipid levels were not significantly associated with diabetic retinopathy. Although more studies are needed to confirm these findings and to elucidate the mechanisms for these associations, our findings support the fact that these clinically available and feasible apolipoprotein measures may be better biomarkers of diabetic retinopathy than traditional lipid measures.

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