EXTENDED REPORT

Plasma apolipoproteins and risk for age related maculopathy

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Aim: To determine if elevated plasma levels of atherogenic and/or anti-atherogenic lipoproteins are risk factors for developing age related maculopathy (ARM).

Methods: In a cross sectional study in a university clinic setting, 129 patients (72 women and 57 men) underwent colour fundus photography, acuity and contrast sensitivity assessment, and electroimmunoassays of plasma apolipoproteins B (apoB) and A-I (apoA-I), the principal proteins of low density and high density lipoproteins, respectively. Maculopathy stage was assigned using the AREDS grading system.

Results: Levels of apoB in no ARM, mild, intermediate, and advanced ARM groups were 93.3, 91.8, 95.2, and 98.2 mg/dl, respectively. Levels of apoA-I were 147.4, 148.6, 141.0, and 144.9 mg/dl in the same groups. There was no significant association between these measures, typical for age, and maculopathy stage.

Conclusion: Although drusen associated with ARM and ageing contain cholesterol and apoB, like the lipid rich core of an atherosclerotic plaque, the results of this study and our previous work in toto make the prospects of a plasma origin for these lesion constituents increasingly untenable. This conclusion is consistent with an emerging hypothesis that a large lipoprotein of intraocular origin is an important pathway for constituent retinal lipid processing and the biogenesis of drusen.

Age related maculopathy (ARM) is the major cause of new vision loss among elderly people in industrialised countries. Despite numerous treatments directed towards the complication of choroidal neovascularisation,^{1–4} and the identification of apparently robust genetic factors including apolipoprotein E (apoE) and complement factor H,^{5–9} causes of the underlying degeneration remain obscure. A critical assessment of evidence for overlapping risk factors for coronary artery disease (CAD) and ARM drew parallels between pathophysiological processes in these two diseases.¹⁰ Prominent ARM lesions involve Bruch's membrane (BrM), a specialised vessel wall separating photoreceptors and their support cells, the retinal pigment epithelium (RPE), from their blood supply. Because drusen and BrM contain abundant lipids, including cholesterol,^{11–18} and because BrM and arterial intima share some structural properties, ARM and CAD may have common mechanisms of cholesterol accumulation in their respective characteristic lesions.

Elevated total plasma cholesterol is a defining risk factor for atherosclerosis.¹⁹ Since 1963, 26 studies measured plasma cholesterol levels in ARM patients, providing largely inconclusive results (table 1; see supplementary table on the *BJO* website (www.bjophthalmol.com/supplemental) for details of patient populations). One explanation is methodological—that is, most studies included few patients or used heterogeneously defined disease stages. Further, most studies used total cholesterol assays which are widely available but do not specify the nature of lipoproteins that carry the cholesterol in plasma. Such assays are therefore considered less informative than measurement of plasma lipoprotein concentrations.

Lipoproteins are submicroscopic particles composed of lipid and protein held together by non-covalent forces. Each particle is a microemulsion consisting of an outer layer of phospholipids (PL), unesterified cholesterol (UC), and apolipoproteins, and a core of neutral lipids, predominantly esterified cholesterol (EC) and triglycerides (TG). Within the essential microemulsion structural motif, lipoprotein classes differ in relative amount of lipids, protein/lipid ratio, and

apolipoprotein species present, resulting in differences in size, density, and electrophoretic mobility. Major plasma lipoprotein classes are very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). Apolipoproteins are amphipathic—that is, they have both hydrophobic and hydrophilic regions that interact with both lipoprotein lipids and the aqueous environment.²⁰ Via amphipathic regions, apolipoproteins, acting as detergents, largely determine and stabilise lipoprotein particle size and structure. Plasma apolipoproteins are grouped into two classes, non-exchangeable (apoB-100 and apoB-48) and exchangeable (apoA-I, apoA-II, apoA-IV, apoC-I, apoC-II, apoC-III, and apoE).²¹

Plasma lipoprotein concentrations figure importantly in CAD development. Elevated concentrations of total cholesterol and LDL cholesterol are associated with increased CAD risk, whereas increased levels of HDL cholesterol are associated with decreased CAD risk.^{22–24} Further, the concentrations of apoA-I, the major apolipoprotein in HDL, and apoB, essentially the only apolipoprotein in LDL, may be better predictors of CAD risk than HDL and LDL cholesterol concentrations.^{24–28} The predictive strength of apoB as a CAD risk factor relates to the fact that each LDL particle has one apoB molecule. Whereas LDL cholesterol content varies, apoB concentration reflects an exact estimate of atherogenic particle number,²⁹ and the apoA-I/apoB ratio is a good indicator of CAD risk.^{25–26}

Another explanation for the weak link between ARM and plasma cholesterol, however, is mechanistic, in that accumulating evidence suggests that the cholesterol in aged BrM and sub-RPE is derived from a lipoprotein of intraocular

Abbreviations: apo, apolipoprotein; AREDS, Age-Related Eye Disease Study; ARM, age related maculopathy; BDES, Beaver Dam Eye Study; BrM, Bruch's membrane; CAD, coronary artery disease; EC, esterified cholesterol; EDCCS, Eye Disease Case Control Study; HDL, high density lipoproteins; LDL, low density lipoproteins; PL, phospholipids; RPE, retinal pigment epithelium; UC, unesterified cholesterol; VLDL, very low density lipoproteins

Table 1 Relation between age related maculopathy (ARM) and atherogenic plasma lipids/lipoproteins

Reference	Assays	Results	OR	CI
Albrink, 1963 ⁶⁷	Tot-C	NS*		
Landolfo, 1978 ⁶⁸	Tot-C, β lipo, pre- β , TG	NS		
Vidaurre, 1984 ⁶⁹	Tot-C, VLDL, LDL, TG	NS		
Blumenkranz, 1986 ⁷⁰	Tot-C, LDL-C, VLDL-C, TG	NS		
Goldberg, 1988 ⁷¹	Tot-C	NS		
EDC-CS Group, 1992 ⁷²	Tot-C, TG	Tot-C $\uparrow$	4.10	2.30 to 7.30
Tsang, 1992 ⁷³	Tot-C, TG	NS		
Klein, 1993 ⁷⁴	Tot-C	Tot-C $\downarrow$, women, early ARM Tot-C $\downarrow$, men, early ARM	0.89 0.66	0.80 to 0.98 0.51 to 0.88
Sanders, 1993 ⁷⁵	Tot-C, TG	NS		
Klein, 1997 ⁷⁶	Tot-C	NS		
Smith, 1998 ⁷⁷	Tot-C, TG	NS		
Klein, 1999 ⁷⁸	Tot-C, LDL-C, apoB	NS		
Klein, 1999 ⁷⁹	Tot-C, LDL-C, TG, apoB	TG $\downarrow$, soft drusen apoB $\uparrow$, exudative	0.88 9.16	0.79 to 0.99 1.82 to 46.25
Hyman, 2000 ⁸⁰	Tot-C, LDL-C, TG	NS		
Delcourt, 2001 ⁸¹	Tot-C, TG, apoB	NS		
Ikeda, 2001 ⁸²	Tot-C, TG, apoB, ox-LDL	ox-LDL $\uparrow$		
Belda Sanchis, 2001 ⁸³	Tot-C, VLDL-C, LDL-C, TG, apoB	Tot-C $\uparrow$, LDL-C, VLDL-C, apoB		
Abalain, 2002 ⁸⁴	Tot-C, TG, apoB, Lp(a)	NS†		
Klein, 2003 ⁸⁵	Tot-C, LDL-C, TG	Tot-C $\downarrow$, early ARM LDL-C $\downarrow$, early ARM TG $\downarrow$, early ARM	0.96 0.96 0.98	0.93 to 0.99 0.93 to 1.00 0.96 to 0.99
Miyazaki, 2003 ⁸⁶	Tot-C, TG	NS		
Wachter, 2003 ⁸⁷	Tot-C, LDL-C, TG, Tot/HDL-C	NS Tot-C, LDL-C, TG; Tot $\uparrow$ /HDL-C		
Wilson, 2004 ⁸⁸	Tot-C, LDL-C, HDL-C, TG	log baseline TG $\uparrow$ associated with CNV	1.45	1.14 to 1.86
Van Leeuwen, 2004 ⁸⁹	Tot-C	NS		
Tomany, 2004 ⁹⁰	Tot-C	Tot-C $\uparrow$, GA Tot-C $\downarrow$, NV	1.08 0.94	1.00 to 1.15 0.88 to 0.99
McGwin, 2005 ⁹¹	Tot-C, LDL-C	Tot-C $\downarrow$, NS LDL-C		
Nowak, 2005 ⁹⁴	Tot-C, LDL-C, TG, apoB, Lp(a)	Tot-C $\uparrow$, LDL-C, TG, apoB, NS Lp(a)		

β lipo, β lipoprotein (LDL); pre- β lipo, pre- β lipoprotein (VLDL); LDL, low density lipoprotein; VLDL, very low density lipoprotein; Lp(a), lipoprotein (a); Tot-C, total cholesterol; LDL-C, LDL cholesterol; VLDL-C, VLDL cholesterol; TG, triglycerides; ApoB, apolipoprotein B; ox-LDL, oxidised LDL; CNV, choroidal neovascularisation; GA, geographic atrophy; NV, neovascular.

*NS, not significant; blank signifies that variable was not measured; OR, odds ratio; CI, confidence interval.

†apo C-III, E decreased significantly.

origin.^{17 18 30–32} If true, then an association with plasma lipoprotein levels is not expected. Therefore, to distinguish between the alternatives of plasma and intraocular origin, we determined circulating apoA-I and apoB concentrations in well characterised patients at different stages of ARM.

METHODS

Patient recruitment and ascertainment

The institutional review board of the University of Alabama at Birmingham approved this study. The research followed the tenets of the Declaration of Helsinki. Informed consent was obtained from all participants after the nature and possible consequences of the study were explained.

Participants were recruited from the comprehensive ophthalmology and retina services of the Department of Ophthalmology, University of Alabama at Birmingham, based on clinic patients seen over a 14 month period from August 2003 to September 2004. This cohort was assembled primarily for the purposes of another investigation³³ and was an opportunity to address the issues described above. Eligibility criteria were as follows: (1) at least 50 years of age; and (2) best corrected distance visual acuity as listed in the medical record of 20/80 or better in at least one eye. Participants were excluded if the medical record or a general health interview indicated that they had any ocular conditions other than AMD, neurological diseases such as Alzheimer's disease, Parkinson's disease, history of stroke, diabetes, or serious frailty. Participants with refractive error ≤ 6 dioptres or dry eye were eligible.

Data collection

Questionnaires were interviewer administered and included a review of demographic (age, sex, race/ethnicity) and

behavioural (smoking) characteristics. General health was estimated through a questionnaire that asked about the presence of medical problems in 17 areas.³³ Subjects were also queried regarding the use of prescription and non-prescription medications. Best corrected distance visual acuity was measured for each eye using the ETDRS chart³⁴ and expressed as logMAR. Contrast sensitivity was assessed for each eye using the Pelli-Robson chart and its standard administration protocol³⁵ and scored with the letter by letter method.³⁶ Stereoscopic colour 30° fundus photographs of both eyes were taken with a FF450 Plus Zeiss fundus camera at the baseline visit after dilatation of the pupil to at least 6 mm. Photographs were evaluated using the Age-Related Eye Disease Study (AREDS) grading system at the University of Wisconsin Reading Center by graders who were masked to participant characteristics. In this system, step 1 indicates normal retinal ageing, steps 2–6 the early phases of ARM, steps 7–9 intermediate ARM, and steps 10–11 advanced disease.³⁷

Apolipoprotein assays

The concentrations of apoA-I and apoB were measured by electroimmunoassays as described^{38 39} using polyclonal antibodies to these apolipoproteins. The monospecificity of these antibodies to human apoA-I and apoB, produced in our laboratory, has been established.^{40–43}

Statistical analysis

ARM presence and severity in the eye with the worse AREDS grading system step was used to define ARM presence and severity for each participant. Demographic, visual function, medical characteristics, and plasma apolipoprotein levels were compared according to ARM severity (none, mild,

Table 2 Demographic, visual function, medical characteristics, and plasma apolipoprotein levels, stratified by ARM severity

	None (n = 32)		Mild (n = 45)		Intermediate (n = 13)		Advanced (n = 39)		p Value
Age, years, mean (SD)	69.4	(5.7)	70.6	(8.9)	74.5	(11.2)	72.9	(8.7)	0.1960
Sex, % (n)									0.8450
Women	59.4	(19)	55.6	(25)	53.9	(7)	53.9	(21)	
Men	40.6	(13)	44.4	(20)	46.2	(6)	46.2	(18)	
Race, % (n)									0.0003
White	93.8	(30)	95.6	(43)	92.3	(12)	100.0	(39)	
African-American	6.3	(2)	4.4	(2)	7.7	(1)	0.0	(0)	
Visual acuity, logMAR, mean (SD)									
Better eye	0.08	(0.11)	0.13	(0.14)	0.16	(0.17)	0.21	(0.25)	0.0314
Worse eye	0.19	(0.14)	0.30	(0.24)	0.41	(0.28)	0.70	(0.37)	0.0001
Contrast sensitivity, mean (SD)									
Better eye	1.41	(0.15)	1.40	(0.15)	1.32	(0.12)	1.31	(0.22)	0.0174
Worse eye	1.41	(0.15)	1.37	(0.19)	1.23	(0.23)	0.93	(0.49)	0.0001
General health, mean (SD)									
No of co-morbid conditions	3.2	(1.7)	3.7	(2.1)	4.1	(2.0)	4.8	(1.6)	0.0068
Smoking, % (n)									0.2692
Never	59.4	(19)	46.7	(21)	15.4	(2)	33.3	(13)	
Previous	34.4	(11)	40.0	(18)	64.5	(8)	59.0	(23)	
Current	6.3	(2)	11.1	(5)	23.1	(3)	7.7	(3)	
Unknown	0.0	(0)	2.2	(1)	0.0	(0)	0.0	(0)	
Lipid lowering medications, % (n)									0.8296
Yes	21.9	(7)	31.1	(14)	30.8	(4)	33.3	(13)	
No	78.1	(25)	68.9	(31)	69.2	(9)	66.7	(26)	
Total cholesterol, mg/dl, mean (SD)	201.0	(33.3)	198.0	(36.6)	194.0	(41.9)	203.1	(36.2)	0.9338
ApoA-I, mg/dl, mean (SD)	147.4	(39.8)	148.6	(30.9)	141.0	(32.2)	144.9	(24.8)	0.8672
ApoB, mg/dl, mean (SD)	93.3	(19.4)	91.8	(20.2)	95.2	(23.5)	98.2	(25.7)	0.7461

intermediate, advanced) using χ^2 tests and analysis of variance for categorical and continuous variables, respectively. p Values ≤ 0.05 (two sided) were considered significant.

RESULTS

Table 2 shows demographics, visual function, medical characteristics, and plasma apoA-I and apoB levels of the 129 study patients. There were no differences between the groups according to age or sex. The advanced ARM group was solely white while the other groups had small representations of African-Americans. Visual acuity and contrast sensitivity in both the better and worse eye worsened with increasing disease severity; visual function of these patients is described fully elsewhere.³³ The intermediate and advanced ARM groups had more co-morbid medical conditions than the other groups. With regard to smoking, never smokers dominated the no and mild ARM group, and previous smokers dominated the intermediate and advanced ARM groups, though these differences were not statistically significant. The frequency of lipid lowering medication use did not differ across the groups.

Total plasma cholesterol was not different across groups and had levels typical for this age group.⁴⁴ Neither apoA-I, apoB, nor the apoA-I/apoB ratio differed significantly among these groups. These values, too, were typical for their age group.⁴⁵

DISCUSSION

Our major finding is that in a sample of well characterised ARM patients neither apoB nor apoA-I levels in plasma were significantly associated with ARM presence or severity as defined by the masked grading of stereo fundus photographs.

A critical juncture in the understanding of the link between plasma lipoproteins and adverse CAD events occurred when a sufficient number of epidemiological studies with strong statistical associations between CAD and elevated levels of plasma cholesterol or atherogenic lipoproteins was amassed (for example, Verschuren *et al*¹⁹ and Walldius *et al*⁴⁶). For reference, it is now established that each

1% decrement in LDL cholesterol is associated with a 1% decrement in risk for myocardial events.⁴⁷ If the apoB and cholesterol rich drusen associated with ARM and ageing represented an ocular manifestation of a systemic atherosclerotic process, then one would predict that a similar link could be forged for ARM. The ARM literature (table 1), however, is dominated by citations to the large associations between neovascularisation and plasma cholesterol (OR = 4.1) in the Eye Disease Case Control Study (EDCCS)⁴⁸ and neovascularisation and apoB (OR = 9.2) in the Beaver Dam Eye Study (BDES).⁴⁹ However, of 26 total studies reporting data on plasma cholesterol and/or atherogenic lipoproteins (table 1), five showed a positive association, three showed a negative association, three showed positive and negative associations depending on ARM parameter, and 15 showed no significant associations. It is not clear why EDCCS and BDES obtained the strong effects they did. Although not the strongest indicator of CAD risk (see Introduction), total plasma cholesterol was one measure employed by the vast majority (24/26) of these investigations, permitting cross study comparison. Many studies had a few ARM cases and used different ARM end points. However, if plasma cholesterol were the major source of cholesterol in BrM and sub-RPE deposits, then a more consistent association across this large number of studies should have been detected despite these two limitations.

Relevant to our investigation of apoA-I, the contribution of anti-atherogenic lipoproteins to ARM has been examined by fewer studies than have examined atherogenic lipoproteins (table 3). The function of HDL, with apoA-I at its backbone, is removal of cellular cholesterol from peripheral tissues to the liver where it is converted to bile salts for secretion, a process termed reverse cholesterol transport.⁵⁰ Since elevated HDL levels are considered protective against cardiovascular disease, it is perhaps surprising that of 20 studies (table 3), seven demonstrated higher risk for ARM among patients with elevated HDL, four showed reduced risk, eight showed no effect, and one showed higher and lower risk depending on the variable examined. If BrM cholesterol reflects solely the activity of HDL mediated reverse cholesterol transport at

Table 3 Relation between age related maculopathy (ARM) and anti-atherogenic plasma lipids/lipoproteins

Reference	Assays	Results	OR	CI
Landolfo, 1978 ⁶⁸	α -lipo	NS*		
Vidaurre, 1984 ⁶⁹	HDL-C	NS		
Klein, 1993 ⁷⁴	HDL-C	HDL-C $\uparrow$, men, early ARM	1.48	1.12 to 1.97
Klein, 1997 ⁷⁶	HDL-C	HDL-C $\uparrow$, exudative	0.57	0.36 to 0.90
Smith, 1998 ⁷⁷	HDL-C	NS		
Klein, 1999 ⁷⁸	HDL-C, apoA-I	NS		
Klein, 1999 ⁷⁹	HDL-C, apoA-I	HDL-C $\uparrow$	1.43	1.13 to 1.82
		apoA-I $\uparrow$	2.06	1.19 to 3.55
Hyman, 2000 ⁸⁰	HDL-C	NV $\uparrow$ v control	2.30	1.10 to 1.47
Delcourt, 2001 ⁸¹	HDL-C	HDL-C $\uparrow$, soft drusen	1.52	1.14 to 2.02
	apoA-I	apoA-I $\uparrow$, soft drusen	1.71	1.18 to 2.47
Ikeda, 2001 ⁸²	apoA-I	NS		
Belda Sanchis, 2001 ⁸³	apoA-I	NS		
Abalain, 2002 ⁸⁴	apoA-I	NS		
	non-B LpA-I, LpC-III	non-B LpA-I $\uparrow$, LpC-III $\downarrow$		
Wachter, 2003 ⁸⁷	HDL-C	HDL-C $\downarrow$ with ARM		
Wilson, 2004 ⁸⁸	HDL-C	$\downarrow$ baseline HDL associated with CNV	0.59	0.36 to 0.96
Van Leeuwen, 2004 ⁸⁹	HDL-C	HDL-C $\uparrow$, esp apoE4	1.16	1.00 to 1.35
Tomany, 2004 ⁹⁰	HDL-C	GA, NS		
		NV, NS		
McGwin, 2005 ⁹¹	HDL-C	NS		
Dasch, 2005 ⁹²	HDL-C	HDL-C $\downarrow$, late v early		
Van Leeuwen, 2005 ⁹³	HDL-C	HDL-C $\uparrow$, late v early		
Nowak, 2005 ⁹⁴	HDL-C, apoA-I	HDL-C $\downarrow$, apoA-I		

α -lipo, α -lipoprotein (HDL); HDL, high density lipoprotein; HDL-C, HDL-cholesterol; ApoA-I, apolipoprotein A-I; CNV, choroidal neovascularisation; GA, geographic atrophy; NV, neovascular.

*NS, not significant; blank signifies that variable was not measured; OR, odds ratio; CI, confidence interval.

the basal RPE, then one might expect elevated HDL to be protective, yet the weight of evidence to date suggests just the opposite. The potential association of ARM with elevated anti-atherogenic lipoproteins, the lack of association with elevated atherogenic lipoproteins, and the protective effect of the apoE4 isoform represent three ways that ARM notably differs from atherosclerotic CAD, despite the commonality of extracellular apoB and cholesterol rich intimal lesions.

The concept that an apoB containing lipoprotein of intraocular origin has an important role in ARM rests on the following evidence^{11-17 30-32 51-55}: (1) native human RPE expresses mRNA transcripts for apoA-I, apoB, apoC-I, apoC-II, and apoE; (2) native RPE expresses microsomal triglyceride transfer protein (required for apoB containing lipoprotein secretion, and deficiency of which causes abetalipoproteinaemia); (3) neutral lipid and 100 nm diameter particles with surface and core structure accumulate with age in BrM; (4) drusen contain neutral lipid, apoA-I, apoB, apoC-I, apoC-II, and apoE; (5) lipoprotein particles isolated from BrM differ from plasma lipoproteins in density and cholesterol profile, and cholesteryl esters in BrM differ in saturated fatty acid content relative to those in sclera and cornea; and (6) in appropriately preserved donor eyes with ARM, basal laminar deposits and basal linear deposits contain solid particles in areas previously thought to contain membranous debris. The idea that BrM lipid derives from retina or RPE rather than the circulation is not new, having been postulated by authors of previous compositional studies.⁵⁶⁻⁵⁷ Nevertheless, the specific mechanism—a secreted lipoprotein—and the hypothesis that it represents an output pathway for fatty acids originating from phagocytosed outer segments³¹ is of recent inception.

The most straightforward method of demonstrating that plasma lipoproteins infuse and bind to BrM extracellular matrix, as it does in arterial intima, would be examination of animal models of diet or genetically induced hypercholesterolaemia.⁵⁸⁻⁶² Using transmission electron microscopic examination of murine posterior segment, such studies revealed either a lack of BrM change or accretion of an electron lucent material (membrane bound and non-membrane bound) that is not yet proved as neutral lipid. In addition, interpretation of studies using transgenic mice expressing isoforms of

human apoB-100 and apoE⁶²⁻⁶⁴ is complicated by RPE expression of these same genes, preventing definitive attribution of effects to plasma lipoproteins of hepatic or intestinal origin. However, Malek *et al* recently showed that older mice expressing human apoE2, E3, or E4 isoforms and consuming a high fat diet accumulate oil red O binding material in the sub-RPE space.⁶⁵ Determining the source of this material in future animal models permitting independent manipulation of RPE and plasma derived lipoproteins will be of great interest.

The results of this study should be interpreted in light of strengths and weaknesses. A strength is the use of fundus photographs evaluated by masked graders to classify disease presence and severity based upon a validated classification scheme. Additionally, the technique for identifying lipoprotein classes is also based upon validated methodology. The sample size was small, particularly for the intermediate and advanced groups, thereby limiting the statistical power of the analyses. However, the distributions of apoA-I and apoB were remarkably similar across groups. Other limitations are the focus on patients whose vision was 20/80 or better in at least one eye and advanced AMD patients who were exclusively white people, restricting the applicability of findings to patients with advanced bilateral AMD and to patients of other ethnic or racial backgrounds, respectively.

A lack of an association between ARM stage and plasma apoA-I and apoB does not prove that these apolipoproteins in sub-RPE lesions originate within the eye. However, showing the absence of an association between ARM and plasma apoA-I and apoB by the scientifically well justified and validated methods used here makes the prospect of a predominantly plasma source¹⁶ increasingly untenable. We note that it is possible that a lipoprotein of RPE origin containing apoB or apoA-I may be eventually detectable in plasma with appropriately sensitive methods, but, like a teardrop dissipated into a pail of seawater, its mass is probably insufficient to increase overall levels. The practical implications of our findings for ARM patients remain to be determined. However, one treatment currently under investigation, rheopheresis, a method of plasma ultrafiltration, is predicated, in part, on the assumption that lipids in BrM and

drusen are derived from plasma and are deposited secondly to impaired choroidal circulation.⁶⁶ While not excluding the potential benefit of reducing other plasma proteins by this invasive procedure, our study indicates that a treatment rationale based on plasma lipoprotein levels should be interpreted cautiously.

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A supplementary table is on the BJO website (www.bjophthalmol.com/supplemental)

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Competing interests: none.

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