Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration

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Abstract The largest risk factor for age-related macular degeneration (ARMD) is advanced age. With aging, there is a striking accumulation of neutral lipids in Bruch’s membrane (BrM) of normal eye that continues through adulthood. This accumulation has the potential to significantly impact the physiology of the retinal pigment epithelium (RPE). It also ultimately leads to the creation of a lipid wall at the same locations where drusen and basal linear deposit, the pathognomonic extracellular, lipid-containing lesions of ARMD, subsequently form. Here, we summarize evidence obtained from light microscopy, ultrastructural studies, lipid histochemistry, assay of isolated lipoproteins, and gene expression analysis. These studies suggest that lipid deposition in BrM is at least partially due to accumulation of esterified cholesterol-rich, apolipoprotein B-containing lipoprotein particles produced by the RPE. Furthermore, we suggest that the formation of ARMD lesions and their aftermath may be a pathological response to the retention of a subendothelial apolipoprotein B lipoprotein, similar to a widely accepted model of atherosclerotic coronary artery disease (Tabas, I., K. J. Williams, and J. Borén. 2007. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation. 116:1832–1844). This view provides a conceptual basis for the development of novel treatments that may benefit ARMD patients in the future.—Curcio, C. A., M. Johnson, J-D. Huang, and M. Rudolf. Apolipoprotein B-containing lipoproteins in retinal aging and age-related macular degeneration. J. Lipid Res. 2010. 51: 451–467.

Supplementary key words retinal pigment epithelium • Bruch’s membrane • drusen • basal deposits • cholesterol • retinyl ester

INTRODUCTION TO OUTER RETINA AND CHOROID, STATEMENT OF PURPOSE

Embryologically part of the central nervous system, the retina (Fig. 1A, B) converts light energy to an electrochemical signal for transmission to the brain through the optic nerve. The 100 million rod and cone photoreceptors, located at the outer surface of the retinal sheet, are supported by the retinal pigment epithelium (RPE). This polarized monolayer serves diverse functions essential for optimal photoreceptor health, including daily phagocytosis of photoreceptor outer segment tips, vitamin A metabolism, maintenance of retinal attachment, and coordination of cytokine-mediated immune protection. The photoreceptor distribution varies considerably across the 1,000-mm² retina, with cone density approaching 200,000/mm² in the fovea and rod density similarly high 2–4 mm away from the foveal center. Surrounding the fovea is a pile-up of inner retinal neurons that anatomically defines the 6 mm-diameter extent of the macula, subtending 21° of visual angle.

Two vascular systems supply the human retina. The inner retinal layers rely on the intrinsic retinal circulation and the photoreceptors and RPE depend on the choroid located external to them (Fig. 1C). About 200–300 µm thick, the choroid has the highest blood flow per unit volume in the body, with 7-fold greater flow in the macula relative to the periphery. The innermost 2–4 µm of the choroid, i.e., subjacent to the RPE, is Bruch’s membrane (BrM, Fig. 1B).

Abbreviations: apo, apolipoprotein; ARMD, age-related macular degeneration; BlamD, basal laminar deposit; BlinD, basal linear deposit; BrM, Bruch’s membrane; CAD, coronary artery disease; CM, chylomicron; EC, esterified cholesterol; HBL, hypobetalipoproteinemia; MTP, microsomal triglyceride transfer protein; OTAP, osmium-tannic acid-paraphenylenediamine; QFDE, quick-freeze/deep-etch; RPE, retinal pigment epithelium; TG, triglyceride; UC, unesterified cholesterol.

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sis by many different mentors whose work encompasses cell biology, biochemistry, physical chemistry, and descriptive and experimental pathology of human tissues and animal models (6–19). In this review, we summarize and contextualize recent work, primarily from our own laboratories, establishing that age-related lipid accumulation in BrM can be accounted for by cholesterol-rich lipoproteins of intraocular origin. Many other ARMD-relevant topics (e.g., cell death, genetics, neovascularization, visual function) have been recently reviewed (20–23). A version of this review tailored to an ophthalmic audience is available elsewhere (24).

INTRODUCTION TO ARMD

Epidemiology and risk factors

The macula, required for high-acuity vision, is vulnerable to ARMD (25). The disease has two forms. An underlying degenerative process (“dry” ARMD) includes pathognomonic extracellular lesions (see next section) and RPE cell death via apoptosis across the macula, resulting in a slow but devastating loss of vision as photoreceptors also die (26–28). Choroidal neovascularization (“wet” ARMD), in which choriocapillaries cross BrM and spread laterally within the plane of these lesions, is an urgent and sight-threatening complication of this process. Clinical ophthalmology has

This five layer extracellular matrix is laid flat along one side of the choriocapillaris, a dense capillary bed (Fig. 2A). Unhindered transport across BrM of nutrients to and metabolites from the RPE is essential for normal vision by the photoreceptors (1, 2). It has been instructive for us to analogize BrM, a wall of a capillary bed, to the intima of a large artery, due to its position between diffusion barriers, thickening throughout adulthood, and extracellular matrix composition (3, 4) (see below).

Age-related macular degeneration (ARMD) is a major cause of vision loss in the elderly of the industrialized world. The largest risk factor for ARMD is advanced age. One of the most prominent age-related changes to the human retina is the accumulation of histochemically detectable neutral lipid in normal BrM throughout adulthood (5). This significant, universal, and poorly understood change to BrM has the potential to have a major impact on physiology of the RPE. Further, this lipid deposition occurs in the same BrM compartment as the pathognomonic extracellular, lipid-containing lesions of ARMD.

Our thinking about how lipids contribute to ARMD has been extensively influenced by the vast knowledge base available for atherosclerotic coronary artery disease (CAD), a condition for which lipoprotein deposition in vessel walls is a well-established causative agent (Fig. 2B,C). We have been educated about a lipoprotein-centered view of atherosclерosis by many different mentors whose work encompasses cell biology, biochemistry, physical chemistry, and descriptive and experimental pathology of human tissues and animal models (6–19). In this review, we summarize and contextualize recent work, primarily from our own laboratories, establishing that age-related lipid accumulation in BrM can be accounted for by cholesterol-rich lipoproteins of intraocular origin. Many other ARMD-relevant topics (e.g., cell death, genetics, neovascularization, visual function) have been recently reviewed (20–23). A version of this review tailored to an ophthalmic audience is available elsewhere (24).
been revolutionized by the recent advent of highly specific inhibitors of vascular endothelial growth factor that, when injected intravitreally, not only retard vision loss but also improve vision in many of the 15% of ARMD patients afflicted with choroidal neovascularization (29). Further, some patients at early to intermediate stages of dry ARMD can benefit from supplementation with micronutrient vitamins and zinc (30).

Of ARMD risk factors (31), advanced age and family history are strongly and consistently related to ARMD across multiple studies. Cigarette smoking, hypertension, and cataract surgery appear to increase the risk of progression to neovascular ARMD in most studies. Other risk factors like obesity and atherosclerotic vascular disease have less consistent findings and weaker associations, and no association exists between ARMD and diabetes or ARMD and plasma cholesterol levels. Linkage studies and genome-wide scans have identified polymorphisms in complement factors H and I, HTRA1, ARMS2, and mitochondrial DNA polymorphism A4917G as risk factors, and complement factor B, C3, and apolipoprotein (apo)E4 as protective factors (32–39).

**Histopathology of extracellular lesions**

ARMD’s characteristic lesions are aggregations of lipid-containing extracellular debris in the RPE/BrM complex (drusen and basal deposits, Fig. 3B,C) that ultimately impact RPE and photoreceptor health (40, 41). Drusen are yellow-white deposits seen behind the RPE in a retinal fundus examination. They are typically classified as “hard” and “soft” on the basis of their borders and the level of risk conferred for advanced disease (higher risk for soft) (42–45). Drusen are defined histologically as focal, dome-shaped lesions between the RPE basal lamina and the inner collagenous layer of BrM. They are found, at least in small numbers, in most older adults (46, 47). Molecular constituents of drusen, virtually all identified during the last decade, include vitronectin, tissue inhibitor of metalloproteinase 3 (TIMP-3), complement factor H, fibrillar and nonfibrillar amyloid, complement component C3, and zinc (33, 48–53). The prominence of complement proteins in drusen in conjunction with associations with sequence variances in complement encoding-genes in ARMD (see previous section) has stimulated the current interest in understanding the alternate complement pathway in retinal disease.

Of importance to this review, drusen contain abundant cholesterol and apolipoproteins. Studies using sudanophilic dyes, filipin, or polarizing microscopy identified neutral lipids and polar lipids in age-related and ARMD-related drusen (3, 54–59)(Fig. 3D,E). All drusen, whether hard or soft, have abundant esterified cholesterol (EC) and unesterified cholesterol (UC). Druse cholesterol assumes different morphologies and distributions, including UC-rich cores and EC-rich lakes, which are thought to reflect different formative processes. The distinctive clefts signifying cholesterol crystals in tissue sections (60) have not been reported in these sub-RPE deposits. Immunoreactivity for multiple apolipoproteins, including B, A-I, C-I, C-II, and E, appears in drusen with frequencies ranging from 100% (apoE) to ~60% (A-I) (57, 61–64). ApoC-III, which is abundant in plasma, is present in fewer drusen (16.6%) than apoC-I (93.1%), which is sparse in plasma, indicating either a specific retention mechanism for plasma-derived apolipoproteins within drusen, or an intraocular source.

Basal deposits are two diffusely distributed lesions associated with ARMD that have distinctly different size, composition, and significance (65) (Fig. 3B,C). Basal lamina deposit (BlamD), between the RPE and its basement membrane, forms small pockets in many older normal eyes or a continuous layer as thick as 15 μm in eyes with ARMD (58, 65, 66). Ultrastructurally, BlamD resembles basement membrane material and contains laminin, fibronectin, and type IV and VI collagen (67–70). Thick BlamD, associated with risk for advanced ARMD (65), is more heterogeneous, containing vitronectin, MMP-7, TIMP-3, C3, and C5b-9 (66) and histochemically detectable EC and UC. These components are possibly in transit from the RPE to BrM and/or drusen (55, 57, 58).
Basal linear deposit (BlinD), between the RPE basement membrane and the inner collagenous layer of BrM, is a thin (0.4–2 µm) lipid-rich layer. Because BlinD is located in the same plane and contains the same material as soft drusen, these lesions are likely alternate forms (layer and lump) of the same entity (71). The principal component of these lesions contains lipid and has been known as membranous debris (42, 65, 72). As viewed by transmission electron microscopy following osmium tetroxide postfixation, membranous debris appears as variably sized, contiguous coils or whorls of uncoated membranes consisting of uni- or multi-lamellar electron dense lines surrounding an electron-lucent center. Although membranous debris is frequently interpreted as vesicles with aqueous interiors, recently evidence indicates that it is not vesicular (see below). Rather, ARMD tissues postfixed with osmium tannic acid-paraphenylenediamine (OTAP) to preserve neutral lipid (73) contain linear tracks of solid particles across BlamD and lipid pools within the layer of BlinD (Fig. 3F). Light microscopic histochemical and ultrastructural studies together suggest that BlinD and soft drusen contents are much more highly enriched in UC than the membranes of surrounding cells (24, 55, 58). On the basis of these and other observations, we proposed to change the name of this major lipid-containing component of ARMD-specific lesions from “membranous debris” to “lipoprotein-derived debris” (24).

NEUTRAL LIPIDS ACCUMULATE WITH AGE IN BRM

Clinical observations on the natural history of fluid-filled RPE detachments in older adults led to the hypothesis by Bird and Marshall (74) that a lipophilic barrier in BrM blocked a normal, outwardly-directed fluid efflux from the RPE. This hypothesis motivated a pivotal laboratory study by Pauliekhoff et al. (5), who used three histochemical stains to identify lipids in eyes from donors aged 1 to 95 years with grossly normal maculas (Fig. 4A–D). Oil red O-binding material localized exclusively to BrM, whereas two other dyes (Bromine Sudan Black B and Bromine-Acetone-Sudan Black B) labeled cells throughout the choroid in addition to BrM. All stains indicated the
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EC was localized and quantified in macula and temporal periphery of 20 normal eyes from age 17 to 92 years (Fig. 4F,G). In the macula, EC was undetectable before age 22 years and then rose linearly throughout adulthood to reach high and variable levels in aged donors. EC was detectable in periphery at roughly 1/7th the level of macula but still increased significantly with age. In the same eyes, UC in macular BrM also increased throughout adulthood, although not as steeply as EC, and it did not increase significantly with age in peripheral retina (not shown).

Also in 2001, Haimovici et al. (56) used hot stage polarizing microscopy (85, 86) to examine the birefringence and melting characteristics of lipids in BrM and sclera (Fig. 4E). EC in tissue sections appears as liquid crystals (“Maltese crosses”) when examined through a polarizing filter. When sections are heated and cooled slowly, liquid crystals melt and reform at characteristic temperatures dictated by the saturation level of the major ester. This study showed that BrM and drusen contained Maltese crosses (Fig. 4E) that melted at a higher temperature than those in sclera. Few birefringent crystals signifying triglyceride (TG) were found in either tissue, and the prominent age-related increase in BrM EC was again detected.

EVIDENCE FOR AN INTRAOCULAR APOB-CONTAINING LIPOPROTEIN

BrM lipoprotein morphology and distribution

Ultrastructural studies through the years revealed numerous small (<100 nm), round electron-lucent spaces...
in BrM of older eyes (for review, see ref. 24). Because these spaces occasionally had a single electron-dense line at their borders, they were frequently described as either membranous or vesicular (i.e., liposomes with aqueous interiors). However, conventional methods of tissue preparation for thin-section transmission electron microscopy extract tissue lipids. The OTAP technique (73) was used to show that BrM vesicles were actually solid and electron dense particles (3, 87). Because evidence presented above indicates that the particles are lipoproteins, this name will be used.

Particles were even more striking when demonstrated by quick-freeze/deep-etch (QFDE), an ultrastructural tissue preparation method that reveals lipids and extracellular matrix in exquisite detail. Similar to freeze fracture but with an etching step that removes frozen water from the tissue (88), QFDE was used to demonstrate lipoprotein accumulation in the aortic wall at the earliest stages of atherosclerosis (89–91). Used to examine BrM, QFDE revealed solid particles accumulating with age that also exhibited a shell and core structure (Fig. 5A) (92, 93). Particles typically varied in size from 60–100 nm but could be as large as 300 nm. Occasionally particles appeared to coalesce with one another. The appearance of particles by QFDE was consistent with their designation as lipoproteins (93). They appear solid and do not etch significantly during the QFDE process, indicating that they contain little water. Particles resembled LDL particles in the aortic intima previously seen by QFDE (89). Particles were found in the same locations in BrM as the lipid-containing particles identified using OTAP (3). Particles were extracted with the Folch reagent for lipid (94). The age-related accumulation of particles within BrM throughout adulthood is consistent with light microscopic and biochemical studies of lipid deposition in this tissue.

Composition of lipids in BrM/choroid and isolated lipoproteins

Determining the composition and morphology of lipids accumulating in BrM can be problematic due to the size and marked cellular heterogeneity of the choroid, which is much thicker than BrM itself. Nevertheless, seven studies assayed lipids from extracts of BrM/choroid with varying degrees of choroid removal using techniques including thin-layer chromatography, gas chromatography, enzymatic fluorimetry, and electrospray ionization (summarized in ref. 24). To avoid potential tissue contamination problems and to provide direct evidence for particles with lipoprotein-like properties, two recent studies used a double, high-salt buffer (95) to release actual lipoproteins from BrM/choroid homogenates (63, 96). From these nine studies total, a consensus for the composition and morphology of BrM neutral lipid-containing structure can be derived. Results obtained with comprehensive assays, results repeated with different assays and/or by different laboratories, consistency with histochemical findings, and consistency between tissue and lipoprotein studies were given greater weight in drawing these conclusions.

The lipid compositions of BrM/choroid and that of BrM lipoproteins isolated from that tissue (Table 1) are very similar, suggesting that appropriate lipoprotein isolation techniques can retain most particle properties. Particles released from BrM exhibit a density similar to plasma VLDL, and they have a spherical particle morphology indicating a neutral lipid core (mean diameter = 66 nm, Fig. 5B). Fractions containing lipoproteins also contain apoB, apoA-I, and apoE, all of which have been identified in BrM by immunohistochemistry. These features, as well as those described below, justify considering the particles seen in situ (see previous section) as genuine lipoproteins.

We note the following salient features of BrM lipid/lipoproteins, and where appropriate, compare results to plasma lipoproteins: 1) RPE/BrM/choroid is much more enriched in neutral lipid/EC than neurosensory retina (3, 97, 98). 2) Relative to macula, peripheral BrM/choroid has neutral lipid with similar fatty acid composition and less EC relative to UC (3, 97). 3) Neutral lipids increase markedly with age relative to PLs, especially after age 60 years (99, 100). 4) Within RPE/BrM/choroid, EC is the prominent neutral lipid, exceeding TG by 4- to 10-fold (tissues) or higher (lipoproteins) (63, 96, 98), a notable departure from plasma apoB lipoproteins in the same size class (Table 1). An early report (100) of high TG content in BrM was not replicated. 5) EC represents 50–60% of the cholesterol detected (Table 1, Table 2) (3, 96, 98). 6) The predominant fatty acids in the neutral lipid/EC fraction are linoleate (18:2, 45.1% for tissues, 41.3% for lipoproteins), olate (18:1, 20.3%, 18.9%), palmitate (16:0, 13.9%, 17.4%), arachidonate (20:4n6, 6.8%, 6.5%), and stearate (18:0, 2.5%, 3.0%) (63, 96, 98). Together these compounds account for 88–89% of the EC detected.
Docosahexaenoate (22:n6, 0.5%, 0.5%), a major mole%. n.d., not determined.

Sphingomyelin and retinyl PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; UC, unesterified cholesterol. Sphingomyelin and retinyl ester are also present in lipoproteins but could not be expressed as mole%. n.d., not determined.

Docosahexaenoate (22:n6, 0.5%, 0.5%), a major component of retinal membranes, is present in minute quantities in BrM (Tables 1, 2) (63, 96–98, 101). In isolated particles, phosphatidylcholine is more abundant than phosphatidylethanolamine by a factor of 1.29, and sphingomyelin, which binds tightly to cholesterol, is 32% lower linoleate (18:2n6) among lipoproteins.

RPE lipid processing

Converging and repeatable evidence from microscopio histochemistry, physical chemistry, ultrastructure, and lipid profiling of tissues and isolated lipoproteins has thus established EC as the primary lipid accumulating with age in BrM. Further, of the major lipid classes, only EC is exclusively localized to BrM, i.e., not also distributed throughout the choroid. High EC concentration within aged BrM is a critical clue to its source and mechanism of deposition. Similar considerations were raised decades ago for atherosclerotic plaques, when seminal studies showed that their EC composition included linoleate-rich EC from insudated plasma lipoproteins and oleate-rich EC within intimal cells (9, 81, 82, 103). The preceding section enumerated several distinct differences between BrM lipoproteins and plasma lipoproteins, suggesting that they are not simply derived from a plasma transudate. If they are instead generated locally, the lipoproteins could represent the oil red O-binding droplets that occasionally appear in RPE (104, 105), released by dying or otherwise stressed RPE cells into BrM. However, relative to the BrM particles, droplets in RPE are much larger (1–2 µm), and they have less EC (105) and more retinyl ester (106).

Expression of lipoprotein pathway genes. mRNA transcripts for both apoE and apoB have been confirmed in human RPE and RPE/choroid preparations (57, 107, 108), with apoE expression levels third behind brain and liver (62). RPE contains mRNA transcripts for apo A-I, C-I, and C-II, but not C-III (57, 64, 109). Full-length apoB protein was localized to native RPE using a specific monoclonal antibody (57, 108). Of significance to the apoB system, apoBEC-1 mRNA is not detectable in human RPE (108).

However, it is present in rat RPE and the rat-derived RPE-J cell line (L. Wang and C. A. Curcio, unpublished observations), along with an apoB-48-like protein immunoprecipitable by anti-rat apoB (96), suggesting that rat RPE expresses both apoB-100 and apoB-48 as does liver in this species. Of major importance to the apoB system is the presence of both mRNA and protein for the large subunit of microsomal triglyceride transfer protein (MTP) within native human RPE. The latter was localized with apoB itself to punctate intracellular bodies, presumably endoplasmic reticulum, within both the RPE and, surprisingly, ganglion cells of the neurosensory retina (108). Mouse RPE expresses both MTP isoforms (110). Dual expression of apoB and MTP signifies that RPE has the capability of secreting lipoprotein particles. RPE also express mRNA transcripts for acyl cholesterol acyltransferase-2 (ACAT-2), one of two cellular cholesterol-esterifying enzymes and the.

### Table 1. Lipid profiles of BrM/choroid, and lipoproteins isolated from BrM and plasma

| Mole% | EC | DG | FA | TG | CL | LYP | PC | PE | PS | UC |
|-------|----|----|----|----|----|-----|----|----|----|----|
| BrM/choroid | 29.8 | 0.7 | 3.6 | 3.0 | 1.5 | 0.6 | 15.4 | 12.6 | 6.0 | 26.7 |
| BrM lipoproteins | 32.4 | 1.7 | 6.3 | 3.3 | 3.2 | 1.8 | 14.2 | 9.5 | 4.6 | 22.9 |
| Plasma CM | 8.0 | 1.1 | 1.3 | 73.5 | n.d. | 0.6 | 8.6 | 1.4 | n.d. | 5.5 |
| Plasma VLDL | 8.2 | 1.2 | 0.9 | 62.0 | n.d. | 0.5 | 15.4 | 1.9 | n.d. | 9.9 |
| Plasma LDL | 49.6 | 0.2 | 1.1 | 5.8 | n.d. | 0.5 | 18.7 | 1.3 | n.d. | 22.8 |

Data modified from reference 96.

**EC, esterified cholesterol; DG, diglyceride; FA, nonesterified fatty acid; TG, triglyceride; CL, cardiolipin; LYP, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; UC, unesterified cholesterol. Sphingomyelin and retinyl ester are also present in lipoproteins but could not be expressed as mole%. n.d., not determined.**

### Table 2. BrM lipoproteins vs plasma apoB-lipoproteins

| Lipoprotein | Diameter, nm | Apolipoproteins | EC/(EC+UC) | EC/TG | PC/PE | SPM/PC |
|-------------|--------------|-----------------|-----------|-------|-------|--------|
| BrM         | 66           | A-I, B-100, E, C-I, -II | 0.58 | 11.32 | 1.29 | 0.70 |
| Chylomicron | >70          | A-I, B-48, C-I, -II, -III | 0.59 | 0.11 | 6.99 | 0.28 |
| VLDL        | 25-70        | B-100, C-I, -II, -III, E | 0.45 | 0.13 | 8.30 | 0.28 |
| LDL         | 19-23        | B-100            | 0.69 | 8.52 | 14.90 | 0.37 |

EC, esterified cholesterol; TG, triglyceride; PC, phosphatidylcholine; PE, phosphatidylethanolamine; UC, unesterified cholesterol; SPM, sphingomyelin. All lipids from references 63 and 96. BrM apolipoproteins from references 63, 64, and 96. Plasma lipoprotein diameters and apolipoproteins from reference 200. Plasma lipoprotein SPM/PC calculated from reference 16.
one more specifically associated with lipoprotein production (111, 112).

These RPE gene expression data provide a basis for redesignating the pigmentary retinopathies of abetalipoproteinemia and hypobetalipoproteinemia as intrinsic retinal degenerations, consistent with the recently expanded role of apoB in mammalian physiology [e.g., (14, 113)]. Mutations of the MTP gene cause a rare autosomal recessive disorder, abetalipoproteinemia (ABL, MIM 200100, Bassen-Kornzweig disease). In addition to absent plasma apoB-lipoprotein (114, 115), ABL features a retinopathy with pigmentary changes, reduced electro-oculogram and electoretinogram signals, and a predilection for angioid streaks (fractured BrM) (116, 117). Truncating mutations of the APOB gene cause hypobetalipoproteinemia (HBL, MIM 107730), a genetically heterogeneous autosomal trait primarily characterized by asymptomatic low plasma LDL (118) and a retinal degeneration (119, 120). That the retinopathies associated with these mutations are only partly alleviated by dietary supplementation with lipophilic vitamins normally carried on plasma apoB-lipoproteins (117) is consistent with the interpretation of intrinsic retinal degenerations.

RPE lipid composition and apolipoprotein secretion. Study of neutral lipid homeostasis in outer retina has been highly focused on understanding the biosynthesis and metabolism of docosahexaenoate, which constitutes 35% of the fatty acids in photoreceptor outer segment phospholipids (123). Classic studies established that this fatty acid is transiently stored in the RPE as newly synthesized, rapidly hydrolysable TG before being recycled across the interphotoreceptor matrix to the neurosensory retina (124, 125).

Although essential, excess cholesterol can be toxic, and pathways for UC and EC release that are well studied in other cells are now known to be operative in the RPE as well. Cultured RPE can secrete 37 kDa apoE into high-density fractions (d = 1.18–1.35 g/ml) (126). These investigators also demonstrated a transfer of radiolabeled docosahexaenoate from outer segment membranes to HDL or lipid-free apoA-I in the medium, presumably by ABCA1-mediated mechanisms (127), although this may represent a minute proportion of total fatty acids in HDL (Table 2). ARPE-19 cells (from human) and RPE-J cells (from rat) secrete EC into a lipoprotein-containing fraction following fatty acid supplementation (oleate for ARPE-19, palmitate for RPE-J) (96, 108). Similar in size (mean, 56 nm) to the particles found in native BrM (see above), they also contain little TG. Importantly, RPE-J cells and medium also contain immunoprecipitable \(^{35}\)S-methionine-labeled apoB in a full-length (512 kDa) form and a lower molecular weight band that may be apoB-48 (96). This definitive assay for protein synthesis and secretion was also used to show secretion from human-derived ARPE-19 cells (128).

To summarize, the data reviewed so far support the hypothesis that the RPE constitutively secretes an apoB-containing lipoprotein particle from its basolateral aspect into BrM for eventual clearance into plasma. This model does not preclude other mechanisms of cholesterol release from RPE (129, 130). We envision a large lipoprotein particle (Fig. 6) in the VLDL size and density class that contains apoB-100, apoA-I, apoE, apoC-I, apoC-II, and possibly other proteins. It is secreted with an EC-rich neutral lipid core. The apoB-containing lipoproteins secreted by cultured RPE cell lines are unusual in several respects compared with plasma apoB-lipoproteins (Fig. 6). Particles are EC-rich despite being as large as TG-rich VLDL. Particles are EC-rich when newly secreted, unlike LDL and HDL, whose composition is achieved by enzymatic remodeling in plasma. Although it is possible that smaller lipoproteins secreted by the RPE (126) accumulate in BrM and fuse together to form large particles, as postulated for LDL in arterial intima (9), the size distribution of particles accumulating with age in BrM is inconsistent with a continuously evolving population.

Source of lipids found in RPE lipoproteins. A signature biological activity in the posterior eye is the renewal afforded by daily ingestion of photoreceptor outer segment tips by the RPE, which has the highest phagocytotic load in the body. Older literature speculated that the age-related accumulation of BrM neutral lipid is related to this activity (131, 132). An initially attractive hypothesis that an apoB-lipoprotein from the RPE could eliminate fatty acids released by lysosomal phospholipases after outer segment phagocytosis (108) is now considered unlikely for several reasons. Phagocytosis is not required for secretion of neutral lipids or apolipoproteins in RPE cell lines (96, 108, 126). Other cells store and transport excess fatty acids as TG, but BrM lipoproteins are not TG-rich (see above). BrM lipoproteins and drusen are highly enriched in EC, which photoreceptors lack (123). BrM lipoproteins and
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An early accumulation that decreased later in life, as if their source had diminished once the elastin layer was obstructed.

In eyes over 60 years of age, accumulated particles filled most of the inter-fibrillar space in the inner collagenous layer, and groups of particles began to appear between this layer and the RPE basal lamina. In eyes over 70 years of age, this process culminates in the formation of the lipid wall (Fig. 8), a dense band 3–4 particles thick external to the RPE basal lamina. This tightly packed layer of space-filling particles displaces the structural collagen fibers at the same location in younger eyes that help bind BrM to the RPE basal lamina. Taken together, these observations suggest that lipoprotein accumulation starts in the elastic layer, backs up into the inner collagenous layer, and eventually forms the lipid wall. This blocks the source of lipoproteins to the outer collagenous layer and explains why the concentration of particles in this layer drops in eyes >60 years of age. Importantly, this lipid wall forms at the same location where BlinD is later seen to develop.

The barrier hypothesis: lipid accumulation and transport through BrM

Unlike age-related accumulation of EC in cornea, sclera, and arterial intima, that which occurs in BrM is apparently unique in its potential impact on fluid and nutrient exchange. Deposition of lipoprotein-derived EC may render BrM increasingly hydrophobic with age and impede transport of hydrophilic moieties between the RPE and choroidal vessels. Two decades ago, Bird and Marshall (74) first introduced the hypothesis of a physical barrier, distinct from the physiological blood-retina barrier (148), that could predispose older individuals to multiple retinal conditions (74, 122, 149). Substantial experimental support for a barrier, obtained in BrM/choroid explants from donor eyes of different ages, demonstrates a reduced hydraulic...
conductivity and permeability to macromolecules of BrM both in aging and in ARMD (150–153). The hydraulic resistivity of BrM (inverse of conductivity) correlates strongly with its lipid content (2). Indeed, the age-related increase in hydraulic resistivity of BrM exactly mirrors that of the age-related increase of histochemically detected EC in BrM (1) (Fig. 9). Hydraulic resistances add when flow-limiting regions are in series. Lipid accumulation in BrM adds a new hydraulic resistance in series with an existing resistance. It cannot be bypassed, as the flow must pass through this layer.

This conclusion, based on correlative data in human tissues, is supported by experimental studies of lipid deposition in a model extracellular matrix, Matrigel™ (154). In this system, the hydraulic conductivity (inverse of resistivity) of Matrigel™ was lowered by >50% when 5% LDL-derived lipids (by weight) were added (Fig. 10). This effect was surprisingly large, much larger than if 5% latex spheres were added to the Matrigel™, and larger than predicted by theoretical considerations of interstitial fluid movement (154).

**Transition from aging (lipid wall) to ARMD (BlinD)**

The striking spatial correspondence of the lipid wall, located between the inner collagenous layer and RPE basal lamina in aged eyes, and BlinD, located in the same compartment in eyes with ARMD, makes the lipid wall a likely direct antecedent to BlinD. Ultrastructural examination of eyes at different ARMD stages (65, 71, 155) can identify transitional forms between lipid wall and BlinD components. Whereas the lipid wall consists of packed lipoproteins of similar size, BlinD in its earliest forms consisted of material resembling fused lipoproteins of irregular size. Throughout adulthood, then, RPE production of apoB lipoproteins for normal physiological transport to

The choriocapillaris may be gradually blocked by their own age-related accumulation in BrM, eventually filling this tissue and resulting in a new layer caused by the backward accumulation of these particles toward the RPE. Once this lipid wall begins to form between the inner collagenous layer and the RPE basal lamina, more lipids preferentially fill this potential space, leading to the formation of BlinD, which is linear because of the geometry of the space containing it. Formation of BlinD may damage RPE cells via decreased transport of nutrients and waste products. The response of the RPE cells to this insult may include production of excessive basal lamina materials, contributing to the formation and local accumulation of BlamD (Fig. 3). Drusen formation likely involves the same processes, with participating nonRPE cells eliciting inflammation, further debris accumulation, and three-dimensional expansion of these deposits (156).

**RESPONSE-TO-RETENTION OF AN INTRAOCULAR APOB LIPOPROTEIN**

The pathology associated with atherosclerotic CAD is widely thought to be initiated by the physiological response-to-retention of lipoproteins in the blood vessel wall (19). Following retention in the inner arterial wall, lipoproteins are prone to oxidation and fusion into products that activate the complement system. Complement activation is chemotactic for monocytes, and complement deposition coincides with cholesterol accumulation (157). Trapped lipoproteins would also be susceptible to sphingomyelinase that generates ceramides, which in turn have untoward effects such as induction of nuclear factor kappa B, stimulation of apoptosis, and other pro-inflammatory events (19).
Our current view of ARMD pathogenesis can be compared with the response-to-retention (19) model of CAD at multiple steps (Fig. 11). In a striking parallel with apoB-lipoprotein-instigated disease in arterial intima, the RPE/BrM complex in aging and ARMD also exhibits accumulation of oxidized lipoproteins, different forms of cholesterol, lipid-rich and structurally unstable lesions, and inflammation-driven downstream events. The antecedents of disease in normal physiology have been highlighted with new information and ideas about lipoprotein particles as a source of extracellular cholesterol, intraocular source(s) of lipoprotein particles, and biological processes that can drive lipoprotein production.

We propose that in younger eyes, lipoprotein particles cross BrM for egress to plasma. With advanced age, transit time across BrM increases due to changes in extracellular matrix, particle character, clearance mechanisms, either alone or in combination, resulting in the accumulation of lipoprotein deposits that oxidized in the high-oxygen chorioidal environment (172). Such oxidation products can contribute to ARMD’s progression to choroidal neovascularization and chorioretinal atrophy (172, 173).

The hypothesis that an RPE-derived apoB-lipoprotein retained in a vascular intima evokes downstream consequences (Fig. 11) also provides a new backdrop for the recently recognized roles of inflammatory proteins and regulators in ARMD (174–176). The alternative complement pathway has been a subject of intense inquiry, because proteins of the complement cascade localize to drusen, and variants in CFH, FB, and C3 genes modulate risk for ARMD (33, 36, 37, 177). For reference, atherosclerotic plaques contain many of the same molecules (157, 178, 179), and sub-endothelial LDL is considered an important activator of complement in incipient plaques.

Like atherosclerotic plaques, drusen and basal deposits feature cholesterol and apoB deposition, and BrM, like arterial intima and other connective tissues, accumulates lipoprotein-derived EC with advanced age. Although knowledge about CAD can provide a powerful conceptual framework for developing new ideas about ARMD pathobiology, RPE physiology, and therapeutic approaches (3, 4), these two complex multi-factorial diseases differ in important and ultimately informative ways, both at the level of the vessel wall and in patient populations. Here, we note key differences between events in BrM and those in arterial intima (4, 24). BrM and intima likely have different major sources of neutral lipid-bearing lipoproteins (RPE vs. liver and intestine via plasma). BrM is very thin and arterial intima is thick. Hemodynamics of the choriocapillaris are different from those of a large muscular artery. BrM is influenced by an endothelium and an epithelium, whereas intima supports an endothelium only. Foam cells congregate early in plaque formation, but macrophages are associated primarily with neovascularization in advanced...

**Fig. 11.** Response-to-retention: ARMD, coronary artery disease. The hypothesized progression of ARMD has many parallels to the Response-to-Retention hypothesis of atherosclerotic coronary artery disease (19), beginning with apoB-lipoprotein deposition in a vessel wall. Reproduced with permission from Progress in Retinal and Eye Research.
ARMD. Smooth muscle cells make up the fibrous cap of plaques but have no defined role in ARMD as yet. Fibrous collagens (types I and III) and elastin are major components of incipient lipid-rich plaque core, and they are present where lipoproteins accumulate in aging BrM but are absent from drusen and BlinD in ARMD. Cholesterol crystals do not occur in BrM or sub-RPE lesions unlike plaque. Capillaries participating in neovascularization invade from different directions relative to the elastic layer (choriocapillaris in ARMD, media in CAD, Fig. 2) (180, 181).

Further, despite the apparent commonality of apoB-initiated disease, key differences between ARMD and CAD also exist at the level of patients and populations. Results of multiple studies seeking associations between measures of elevated plasma cholesterol and ARMD, or HDL levels and ARMD, have been largely inconclusive (for review, see ref. 182). ARMD is not considered to be associated with type 2 diabetes (31, 183), a major CAD risk factor. Observational and retrospective studies of plasma lipid-lowering treatments (e.g., statins) in ARMD patients have had decidedly mixed effects (184). The apoE4 genotype is well established as a modest but consistent risk factor for CAD, conferring decreased longevity and increased mortality (185), yet it surprisingly protects against ARMD, reducing risk by 40% (61, 186). One model invokes the opposing effect of cellular cholesterol export from RPE into BrM and from macrophages into intima to explain the opposite direction of apoE4’s influence in ARMD and CAD (24).

CONCLUSIONS AND FUTURE DIRECTIONS

Recent work has now strongly implicated the RPE as a secretor of EC-rich apoB lipoproteins that normally function to remove cholesterol from RPE cells, but when retained in BrM with age contributes importantly to impeding RPE- and photoreceptor function and to forming the specific lesions of ARMD. The conceptual framework, borrowed heavily from decades of atherosclerosis research, provides a wide knowledge base and sophisticated clinical armamentarium that can be readily exploited for the ultimate benefit of ARMD patients. Tools, techniques, and experimental approaches borrowed from the study of CAD should be applied to the eye in order to address critical questions in the near term.

The goal of new therapeutic approaches for ARMD may be best served by a comprehensive understanding of the RPE as a polarized secretor of lipoproteins. The least understood portion of Fig. 11 is the biological function(s) of the postulated apoB system. Elsewhere, we speculated that plasma lipoproteins taken up by RPE are stripped of nutrients required by photoreceptors (e.g., xanthophylls, cholesterol, vitamin E) and repackaged for secretion in BrM as large, EC-rich apoB-lipoproteins (24). Lipoprotein secretion by RPE cells could dispose of excess UC and forestall toxicity in concert with recycling of EC and retinoids (187) back to plasma. Within the RPE, apically-directed recycling of docosahexaenoate to the retina (124) may thus be accompanied by an independent, basally-directed recycling of plasma lipoproteins repackaged for egress to the systemic circulation across BrM. Fortunately, ample work on cell culture systems developed for atherosclerosis and diabetes research can provide expert guidance. Essential questions include identifying the full range of input lipids to an RPE lipoprotein, identifying the full range of lipid egress pathways from the RPE, identifying how an RPE apoB-lipoprotein compares to those from other well-characterized apoB secretors, and untangling the relationship of apical and basal lipid transport systems (109, 124, 188). Recently developed high-fidelity polarized RPE cultures systems (189) will be essential for answering these questions.

The macula is unique to humans and other primates, and only macaque monkeys are known to accumulate neutral lipid in BrM (105) and apoE in drusen (190). A short-lived species that also displays these characteristics and permits in vivo tests of causation remains a high priority research goal. Eyes of established mouse models of atherosclerosis have already been examined in pursuit of that goal. As reviewed elsewhere (24), at least 10 studies using mice with genetic manipulation of apoB or apoE pathways, some in combination with LDL-receptor deficiency, have been published. Some of the most promising models exhibit histochemically and/or ultrastructurally detectable neutral lipid deposits in BrM, RPE degeneration, and upregulation of pro-angiogenic factors (110, 191–194). Interpretation of studies using transgenic mice expressing lipoprotein-related genes are hindered by RPE expression of the same genes, complicating definitive attribution of effects to plasma lipoproteins of hepatic/intestinal origin or to lipoproteins of RPE origin. Furthermore, the effects described in these mouse models occur in the setting of hyperlipidemia, which is not associated with ARMD pathogenesis. Future animal models that independently manipulate RPE- and plasma-derived lipoproteins, e.g., by using Cre-loxP technology to effect tissue-specific gene deletion (195, 196), will thus be highly informative.

We anticipate that the model we presented for BrM lipoprotein accumulation may facilitate the construction of improved in vitro model systems to study transport across natural and artificial matrices. Such systems could use readily available LDL as an acceptable surrogate for BrM lipoproteins because of its high EC content. Use of such systems have already demonstrated that LDL can pass through bovine BrM (197), although slowed in transit, and that LDL deposition significantly decreases the hydraulic conductivity of an extracellular matrix (154). Future studies focused on determining why lipid first begins to accumulate in BrM, the mechanism by which this occurs, and how this accumulation impedes transport will be guided by past work on factors influencing lipoprotein modification and aggregation in the arterial intima (e.g., ref. 198).

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REFERENCES

1. Ethier, C. R., M. Johnson, and J. Ruberti. 2004. Ocular biomechanics and biotransport. Ann. Rev. Biomed. Eng. 6: 249–273.

2. Marshall, J. A. A. Hussain, C. Starita, D. J. Moore, and A. L. Patmore. 1998. Aging and Bruch’s membrane. In The Retinal Pigment Epithelium: Function and Disease. M. F. Marmor and T. J. Wollenberger, editors. Oxford University Press, New York. 669–692.

3. Curcio, C. A., C. L. Millican, T. Bailey, and H. S. Kruth. 2001. Accumulation of cholesterol with age in human Bruch’s membrane. Invest. Ophthalmol. Vis. Sci. 42: 265–274.

4. Sivaprasad, S. A., C. L. Millican, T. Bailey, and H. S. Kruth. 2001. Association of apolipoprotein E epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. Am. J. Ophthalmol. 135: 253–359.

5. Congdon, N. G., B. O’Colmain, C. C. Klaver, R. Klein, B. Munoz, D. S. Friedman, J. Kempen, H. R. Taylor, and P. Mitchell. 2004. Causes and prevalence of visual impairment among adults in the United States. Arch. Ophthalmol. 122: 477–485.

6. Del Priore, L. V., Y. H. Kuo, and T. H. Zeel. 2002. Age-related changes in human RPE cell density and apoptosis proportion in situ. Invest. Ophthalmol. Vis. Sci. 43: 3312–3318.

7. Sung, K. T., C. T. Dght, G. S. Ying, and A. H. Milam. 2002. The role of apoptosis in age-related macular degeneration. Arch. Ophthalmol. 120: 1435–1442.

8. Curcio, C. A., N. E. Medeiros, and C. L. Millican. 1996. Photoreceptor loss in age-related macular degeneration. Invest. Ophthalmol. Vis. Sci. 37: 1296–1299.

9. Ciulla, T. A., and P. J. Rosenfeld. 2009. Anti-vascular endothelial growth factor therapy for neovascular age-related macular degeneration. Curr. Opin. Ophthalmol. 20: 158–165.

10. Age-Related Eye Disease Study Research Group. 2001. A randomized, placebo-controlled, clinical trial of high-dose supplementation with vitamins C and E, beta carotene, and zinc for age-related macular degeneration and vision loss. AREDS Report No. 8. Arch. Ophthalmol. 119: 1417–1436.

11. Klein, R., T. Petro, A. Bird, and M. R. Vanwesik. 2004. The epidemiology of age-related macular degeneration. Am. J. Ophthalmol. 137: 486–495.

12. Souied, E. H., P. Benlian, P. Amouyel, J. Feingold, J. P. Lagarde, A. Mimnich, J. Kaplan, G. Coscas, and G. Soubrene. 1998. The epsilon4 allele of the apolipoprotein E gene as a potential protective factor for exudative age-related macular degeneration. Am. J. Ophthalmol. 125: 353–359.

13. Sounds, D. G. M., R. T. Petro, A. Bird, and M. R. Vanwesik. 2004. The epidemiology of age-related macular degeneration. Am. J. Ophthalmol. 135: 253–259.

14. Blau, R. J., and J. P. Kane. 2001. Introduction: structure and metabolism of plasma lipoproteins. In The Metabolic and Molecular Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 2707–2716.

15. Segrest, J. M., K. Jones, H. De Loof, and N. Dashti. 2001. Structure of apolipoprotein B-100 in low density lipoproteins. J. Lipid Res. 42: 1346–1367.

16. Björkergen, J. M., V. Veniant, M. A. Sullivan, N. H. Zlot, B. Björkergen, L. B. Nielsen, J. S. Wong, R. L. Hamilton, and S. G. Young. 1999. Analysis of the role of microsomal triglyceride transfer protein in the liver of tissue-specific knockout mice. J. Clin. Invest. 103: 1287–1298.

17. Havel, R., and J. P. Kane. 2001. Introduction: structure and metabolism of plasma lipoproteins. In The Metabolic and Molecular Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, New York. 2707–2716.

18. Jonas, A. 2002. Lipoprotein structure. In Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. E. Vance, editors. Elsevier, Amsterdam. 483–504.

19. Luiss, A., A. M. Fogelman, and G. C. Fonarow. 2004. Genetic basis of atherosclerosis: part I: new genes and pathways. Circulation. 110: 1868–1873.

20. Shoulders, C. C., and G. S. Shelnass. 2005. Current biology of MTP: implications for selective inhibition. Curr. Top. Med. Chem. 5: 283–300.

21. Tabas, I., K. J. Williams, and J. Boren. 2007. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation. 116: 1832–1844.

22. Sekar, G. R., C. A. Curcio, K. R. Sloan, and C. Owsley. 2005. Photoreceptor degeneration in aging and age-related maculopathy. In Macular Degeneration. P. L. Penfold and J. M. Provis, editors. Springer-Verlag, Berlin. 45–62.

23. Montezuma, S. R., L. Sobrin, and J. M. Seddon. 2007. Review of genetics in age-related macular degeneration. Semin. Ophthalmol. 22: 229–240.

24. Edwards, A. O., and G. Malek. 2007. Molecular genetics of AMD and current animal models. Angiogenesis. 10: 119–132.

25. Griess, S., and O. Tata. 2008. The role of vascular endothelial growth factor and other endogenous interplayers in age-related macular degeneration. Progr. Retin. Eye Res. 27: 372–390.
43. Klein, R. M. D. Davis, Y. L. Magli, P. Segal, B. E. K. Klein, and L. Hubbard. 1991. The Wisconsin Age-Related Maculopathy Grading System. *Ophthalmology*. 98: 1128–1134.

44. Davis, M. D., R. E. Gangnon, L. Y. Lee, L. D. Hubbard, B. E. Klein, R. Klein, F. L. Ferris, S. B. Bressler, and R. C. Milton. 2005. The Age-Related Eye Disease Study severity scale for age-related macular degeneration: AREDS Report No. 17. *Arch. Ophthalmol.* 123: 1484–1498.

45. Klein, R., B. E. Klein, M. D. Knudtson, S. M. Meuer, M. Swift, and R. E. Gangnon. 2007. Fifteen-year cumulative incidence of age-related macular degeneration: the Beaver Dam Eye Study. *Ophthalmology*. 114: 235–242.

46. Klein, R., B. E. Klein, K. L. P. Linton. 1992. Prevalence of age-related maculopathy. *Ophthalmology*. 99: 933–943.

47. van der Schaft, T. L., C. M. Mooy, W. C. de Bruijn, F. G. Oron, G. Malek, G. Li, C-M. Guidry, N. E. Medeiros, and C. A. Curcio. 2003. Ultrastructural discrimination of lipid droplets and vesicles in human Bruch’s membrane. *Am. J. Pathol.* 159: 329–328.

48. Grobbee, D. E., C. van Broeckhoven, and P. T. de Jong. 1998. Genetic association of apolipoprotein E with age-related macular degeneration. *Exp. Eye Res.* 67: 772–780.

49. Li, C-M., M. Clark, M. Rudolf, and C. A. Curcio. 2007. Distribution of intracellular and extracellular cholesteryl esters associated with aging and age-related macular degeneration. *Arch. Ophthalmol.* 99: 278–286.

50. Hageman, G. S., R. G. Mullins, S. R. Russell, L. V. Johnson, and D. H. Anderson. 1999. Vitronec tin is a constituent of ocular drusen and the vitronectin gene is expressed in human retinal pigmented epithelial cells. *FASEB J.* 13: 477–484.

51. Fariss, R. N., S. S. Apte, B. R. Olsen, K. Iwata, and A. H. Milam. 2007. Distribution of matrix metalloproteinase-9 in drusen and basal deposits of eyes with age-related maculopathy. *Invest. Ophthalmol. Vis. Sci.* 48: 968–977.

52. Löffler, K. U., and W. R. Lee. 1986. Basal linear deposit in the human macula. *Graefes Arch. Clin. Exp. Ophthalmol.* 224: 493–501.

53. Marshall, G. E., A. G. P. Konstas, G. G. Reid, J. G. Edwards, and W. R. Lee. 1992. Type IV collagen and laminin in Bruch’s membrane and basal linear deposit in the human macula. *Br. J. Ophthalmol.* 76: 607–614.

54. Knupp, C., M. P. Munro, P. K. Luther, E. Ezra, and J. M. Squire. 2000. Structure of abnormal muscle cell bodies (collagen VI) associated with human full thickness muscle defects. *J. Struct. Biol.* 129: 38–47.

55. Bird, A. C., and J. Marshall. 1986. Retinal pigment epithelial changes of the eye. *Am. J. Pathol.* 150: 1295–1298.

56. Klein, R., B. E. Klein, M. D. Knudtson, S. M. Meuer, M. Swift, and R. E. Gangnon. 2007. Fifteen-year cumulative incidence of age-related macular degeneration: AREDS Report No. 17. *Arch. Ophthalmol.* 123: 1484–1498.

57. van der Schaft, T. L., C. M. Mooy, W. C. de Bruijn, F. G. Oron, G. Malek, G. Li, C-M. Guidry, N. E. Medeiros, and C. A. Curcio. 2003. Ultrastructural discrimination of lipid droplets and vesicles in human Bruch’s membrane. *Am. J. Pathol.* 159: 329–328.

58. Bird, A. C., and J. Marshall. 1986. Retinal pigment epithelial changes of the eye. *Am. J. Pathol.* 150: 1295–1298.

59. Löffler, K. U., and W. R. Lee. 1986. Basal linear deposit in the human macula. *Graefes Arch. Clin. Exp. Ophthalmol.* 224: 493–501.

60. Marshall, G. E., A. G. P. Konstas, G. G. Reid, J. G. Edwards, and W. R. Lee. 1992. Type IV collagen and laminin in Bruch’s membrane and basal linear deposit in the human macula. *Br. J. Ophthalmol.* 76: 607–614.

61. Knupp, C., M. P. Munro, P. K. Luther, E. Ezra, and J. M. Squire. 2000. Structure of abnormal muscle cell bodies (collagen VI) associated with human full thickness muscle defects. *J. Struct. Biol.* 129: 38–47.

62. Bird, A. C., and J. Marshall. 1986. Retinal pigment epithelial changes of the eye. *Am. J. Pathol.* 150: 1295–1298.

63. van der Schaft, T. L., C. M. Mooy, W. C. de Bruijn, F. G. Oron, G. Malek, G. Li, C-M. Guidry, N. E. Medeiros, and C. A. Curcio. 2003. Ultrastructural discrimination of lipid droplets and vesicles in human Bruch’s membrane. *Am. J. Pathol.* 159: 329–328.

64. Bird, A. C., and J. Marshall. 1986. Retinal pigment epithelial changes of the eye. *Am. J. Pathol.* 150: 1295–1298.

65. van der Schaft, T. L., C. M. Mooy, W. C. de Bruijn, F. G. Oron, G. Malek, G. Li, C-M. Guidry, N. E. Medeiros, and C. A. Curcio. 2003. Ultrastructural discrimination of lipid droplets and vesicles in human Bruch’s membrane. *Am. J. Pathol.* 159: 329–328.
103. Smith, E. B., P. H. Evan, and M. D. Downham. 1967. Lipid in the
104. Tamminen, M., G. Mottino, J. H. Qiao, J. L. Breslow, and J. S. Frank.
105. Nievelstein, P. F., A. M. Fogelman, G. Mottino, and J. S. Frank.
106. Anderson, M. D., W. W. Dawson, J. Martinez-Gonzalez, and C. A. Curcio.
107. Redmond, T. M., S. Yu, E. Lee, D. Bok, D. Hamasaki, N. Chen, P. Goletz, J.-X. Ma, R. K. Crouch, and K. Pfeiffer. 1998. Rpe65 is neces-
108. Mullins, R. F., S. R. Russell, D. H. Anderson, and G. S. Hageman. 2006. Drusen associated with aging and age-related macular degener-
109. Frank, J. S., and A. M. Fogelman. 1989. Ultrastructure of the
110. Huang, J.-D., B. H. Chung, J. B. Presley, C. L. Millican, and N. E. Medeiros.
111. Sheraidah, G., R. Steinmetz, J. Maguire, D. Pauleikhoff, J. Marshall,
112. Chung, B. H., G. Tallis, V. Yalamoori, G. M. Anantharamaiah, and J. P. Segrest. 1994. Liposome-like particles isolated from hu-
113. Madsen, E. M., M. L. Lindegaard, C. B. Andersen, P. Damm, and L. B. Nielsen. 2004. Human placenta secretes apolipoprotein B containing lipoprotein particles. J. Lipid Res. 45: 3231–3237.
114. Wetterau, J. R., L. P. Aggerbeck, M. E. Bouna, C. Eisenberg, A. Munk, M. Hermier, J. Schmitz, G. Gay, D. J. Rader, and E. G. Gregg. 1992. Absence of microsomal triglyceride transfer protein in individuals with abetalipoproteinemia. Science. 258: 999–1001.
115. Berriot-Varouxaux, N., L. P. Aggerbeck, M. Samson-Bouna, and J. R. Wetterau. 2000. The role of the microsomal triglycer-
116. Gorin, M. B., T. O. Paul, and D. J. Rader. 1994. Angioid streaks associated with abetalipoproteinemia. Ophthalmic Genet. 15: 151–159.
117. Chowers, L., E. Banin, S. Merin, M. Cooper, and E. Granot. 2001. Long-term assessment of combined vitamin A and E treatment for the prevention of retinal degeneration in abetalipoproteinemia patients. Eye. 15: 525–530.
118. Schonfeld, G. 2005. Familial hypobetalipoproteinemia: a review. Curr. Opin. Lipidol. 16: 225–230.
119. Talmud, P. J., C. Converse, E. Kruit, L. Hug, G. G. McIlwaine, J. J. Series, P. Boyd, G. Schonfeld, A. Dunning, and S. Humphries. 1992. A novel truncated apolipoprotein B (apo B55) in a patient with familial hypobetalipoproteinemia and atypical retinitis pigmentosa. Clin. Genet. 42: 62–70.
120. Yee, R. D., P. N. Herbert, D. R. Bergsma, and J. J. Biemer. 1976. Anypical retinitis pigmentosa in familial hypobetalipoproteinemia. Am. J. Ophthalmol. 82: 64–71.
121. Brosnahan, D. M., S. M. Kennedy, C. A. Conover, W. R. Lee, and H. M. Hammer. 1994. Pathology of hereditary retinal degener-
122. Konn, C. A., S. G. Jacobson, A. V. Cideciyan, Z-Y. Li, E. M. Stone, D. Possin, and A. H. Milam. 1996. Sub-retinal pigment epithelial deposits in a dominant late-onset retinal degeneration. Invest. Ophthalmol. Vis. Sci. 37: 1772–1782.
123. Fliers, S. J., and R. E. Anderson. 1983. Chemistry and metabo-
124. Bazan, N. G., W. C. Gordon, and E. B. Rodriguez de Turco. 1992. Docosahexaenoic acid uptake and metabolism in photoreceptors: retinal conservation by an efficient retinal pigment epithelial cell-mediated recycling process. Adv. Exp. Med. Biol. 319: 295–306.
125. Rodriguez de Turco, E. B., N. Parkins, A. V. Ershov, and N. G. Bazan. 1999. Selective retinal pigment epithelial cell lipid metabo-
126. Retinal lipoproteins and age-related macular degeneration
171. Joffre, C., L. Leclere, B. Butecu, L. Martine, S. Cabaret, L. Malbitte, N. Acar, G. Lizard, A. Bron, C. Creuzot-Garcher, et al. 2007. Oxysterols induced inflammation and oxidation in primary porcine retinal pigment epithelial cells. Curr. Eye Res. 32: 271–280.
172. Moreira, E. F., I. M. Larrayoz, J. W. Lee, and I. R. Rodriguez. 2009. 7-Ketocholesterol is present in lipid deposits in the primate retina: potential implication in the induction of VEGF and CNV formation. Invest. Ophthalmol. Vis. Sci. 50: 525–532.
173. Tamai, K., R. F. Spaide, A. E. Ellis, S. Iwabuchi, Y. Ogura, and D. Armstrong. 2002. Lipid hydroperoxide stimulates subretinal chorioidal neovascularization in the rabbit. Exp. Eye Res. 74: 301–308.
174. Hageman, G. S., P. J. Luthert, N. H. C. Chong, L. V. Johnson, D. H. Anderson, and R. F. Mullins. 2001. An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch’s membrane interface in aging and age-related macular degeneration. Prog. Retin. Eye Res. 20: 705–732.
175. Nussenblatt, R. B., and F. Ferris 3rd. 2007. Age-related macular degeneration. Adv. Immunol. 90: 496–503.
176. Richards, A., D. Kavanagh, and J. P. Atkinson. 2007. Inherited complement regulatory protein deficiency predisposes to human disease in acute injury and chronic inflammatory states the examples of vascular damage in atypical hemolytic uremic syndrome and debris accumulation in age-related macular degeneration. Adv. Immunol. 96: 141–177.
177. Johnson, I., V. S. Ozaki, M. K. Staples, P. A. Erickson, and D. H. Anderson. 2000. A potential role for immune complex pathogenesis in drusen formation. Exp. Eye Res. 70: 441–449.
178. Oksjoki, R., P. T. Kovannen, S. Meri, and M. O. Pentikainen. 2007. Function and regulation of the complement system in cardiovascular diseases. Front. Biosci. 12: 4696–4708.
179. Torzewski, M., M. Klouche, J. Hock, M. Messner, B. Dorweiler, J. Torzewski, H. E. Gabbert, and S. Bhakdi. 1998. Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the dyslipidemia in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 18: 369–378.
180. Grossniklaus, H. E., and W. R. Green. 2004. Choroidal neovascularization. Am. J. Ophthalmol. 137: 496–503.
181. Stary, H. C., A. B. Chandler, R. E. Dinsmore, V. Fuster, S. Glagov, W. J. Insull, Jr., M. E. Rosenfeld, C. J. Schwartz, W. D. Wagner, and R. W. Wissler. 1995. A definition of advanced types of atherosclerotic lesions and a histological classification of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Circulation. 92: 1355–1374.
182. Dashni, N., G. McGwin, Jr., C. Owsley, and C. A. Curcio. 2006. Plasma apolipoproteins and risk for age-related maculopathy. Br. J. Ophthalmol. 90: 1028–1033.
183. Adili, M., S. O. Ololsson, M. R. Taskinen, and J. Borén. 2008. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidaemia in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 28: 1225–1236.
184. Chuo, J. Y., M. Wiens, M. Etminan, and D. A. Maberley. 2007. Use of lipid-lowering agents for the prevention of age-related macular degeneration: a meta-analysis of observational studies. Ophthalmic Epidemiol. 14: 367–374.
185. Smith, J. D. 2002. Apolipoproteins and aging: emerging mechanisms. Aging Res. Rev. 1: 345–365.
186. Thakkinian, A., S. Bowe, M. McEvoy, W. Smith, and J. Attia. 2006. Association between apolipoprotein E polymorphisms and age-related macular degeneration: a HuGE review and meta-analysis. Am. J. Epidemiol. 164: 813–822.
187. Quishat, N. M., T. M. Redmond, and D. R. Pepperberg. 2003. Acute radiolabeling of retinoids in eye tissues of normal and apoe-/- deficiency mice. Invest. Ophthalmol. Vis. Sci. 44: 1435–1446.
188. Torzewski, H. E. Gabbert, and S. Bhakdi. 1998. Immunohistochemical demonstration of enzymatically modified human LDL and its colocalization with the terminal complement complex in the dyslipidemia in the metabolic syndrome. Arterioscler. Thromb. Vasc. Biol. 18: 369–378.