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Choroidal and sub-retinal pigment epithelium caverns: multimodal imaging and correspondence with Friedman lipid globules

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Abstract

Purpose: Hyporeflective caverns in eyes with geographic atrophy (GA) secondary to age-related macular degeneration (AMD) and other retinal diseases.

Design: Histological survey of donor eyes with and without AMD. Clinical case series with multimodal imaging analysis.

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Participants: Donor eyes (n=139; 26 early AMD, 13 GA, 40 nvAMD, 52 healthy maculas, and 8 other/ unknown). Forty-one eyes of 28 subjects with GA (16), nvAMD (8), Stargardt disease (4), cone dystrophy (2), pachychoroid spectrum (6), choroidal hemangioma (1), and healthy eyes (4).

Methods: Donor eyes were prepared for macula-wide epoxy resin sections through the fovea and perifovea. In patients, caverns were identified as non-reflective spaces in OCT. Multimodal imaging included color and red free fundus photography, fundus autofluorescence, fluorescein and indocyanine green angiography, OCT-angiography, near-infrared reflectance and confocal multispectral (MultiColor) imaging (Spectralis, Heidelberg Engineering, Germany).

Main Outcome Measures: Presence and morphology of globules. Presence and appearance on multimodal imaging of caverns.

Results: Globules were found primarily in the inner choroidal stroma (91.0%) but also localized to the sclera (4.9%) and neovascular membranes (2.1%). Mean diameters of solitary and multilobular globules were $58.9 \pm 37.8 \mu\text{m}$ and $65.4 \pm 27.9 \mu\text{m}$, respectively. Globules showed morphologic signs of dynamism including pitting, dispersion, disintegration, and crystal formation. Evidence for inflammation in the surrounding tissue was absent. *En face* OCT rendered sharply delimited hyporeflective areas as large as choroidal vessels, frequently grouped around choroid vessels or in the neovascular tissue. Cross-sectional OCT revealed a characteristic posterior hypertransmission. OCT-A showed absence of flow signal within caverns.

Conclusions: Based on prior literature documenting OCT signatures of tissue lipid in atheroma and neovascular AMD, we speculate that caverns are lipid-rich. Globules, with similar sizes and tissue locations in AMD and healthy subjects, are candidates for histologic correlates of caverns. The role of globules in chorioretinal physiology, perhaps as a lipid depot for photoreceptor metabolism, is approachable through clinical imaging.

Précis

Caverns (non-reflective spaces with hypertransmission tails) appear in the choroid and sub-retinal pigment epithelium compartment of healthy and diseased eyes. Localization, demographics, prevalence, and optics, caverns appear to correspond to histologic Friedman lipid globules.

Keywords

age-related macular degeneration; central serous chorioretinopathy; choroid; choroidal caverns; choroidal hemangioma; Friedman lipid globules; cone dystrophy; geographic atrophy; neovascularization; optical coherence tomography; optical coherence tomography angiography; pachychoroid; retinal pigment epithelium; Stargardt disease.

Introduction

Our understanding of the choroid's role in health and disease has expanded greatly thanks to clinical imaging technologies based on optical coherence tomography (OCT), especially with techniques such as enhanced depth imaging, *en face* rendering of chorioretinal structure, swept-source OCT, and OCT-angiography (OCT-A). Information from spectral domain OCT (SD-OCT) and from angiography (dye-and OCT-based) together generated many new concepts including the topography¹ and diurnal variation of choroidal thickness,²

to name just a few. Further, information from a multimodal imaging approach can now be merged to bring the advantages of each individual technique to bear on a single question, resulting in a more comprehensive understanding. Such capability is important for exploring the multiple cell types and functions represented in the choroidal ecosystem.

Histopathology has revealed aspects of choroidal biology that remain to be explored in imaging. In 1934, Jaensch demonstrated that fatty deposits were present in choroidal stroma of eyes of various ages and disease states.³ In 1966, Ephraim Friedman described and illustrated extracellular globules of sudanophilic and osmophilic lipid in a large series of post-mortem eyes, many of which were normal.⁴ In all eyes, choroidal stroma surrounding the globules lacked signs of inflammation.

Clinically, in the choroid of eyes with geographic atrophy secondary to age-related macular degeneration (AMD), Querques et al described caverns by SD-OCT, OCT-A, and indocyanine green angiography (ICGA).⁵ Caverns were defined as hyporeflective, angular-to-round, empty features resembling cavities with punctate/linear internal hyperreflectivity, variably localized in the Sattler and Haller layers, with relative preservation of the choriocapillaris. Due to the absence of a hyperreflective boundary, hyperfluorescence on ICGA, and flow signal on OCT-A, caverns were suggested as “non-perfused ghost vessels and stromal pillars”.⁵ This hypothesis was based on histologic studies^{6,7} demonstrating that choriocapillaries can lose functional endothelium and become ghost vessels, i.e., spaces between the intercapillary pillars of Bruch’s membrane. These spaces in turn may fill with macrophages removing dead endothelium. To explain the appearance of caverns, the authors suggested that similar changes occur in the larger vessels of the Sattler and Haller layers, with the internal punctate hyperreflectivity corresponding to cells. These investigators subsequently described choroidal round hyporeflectivities,⁸ which were similar in size to choroidal caverns, yet distinct due to hyperreflective borders and a consistent round shape, and suggested these as non-or hypo-perfused vessels, single large cells, or cavern precursors. Among the explanations for choroidal round hyporeflectivities of SD-OCT, the authors also included Friedman lipid globules, citing a preliminarily presented survey of these features.⁹

Independently, we observed SD-OCT signatures similar to caverns⁵ not only in the choroid of several retinal conditions but also in the sub-retinal pigment epithelium (sub-RPE) space in neovascular AMD. We thus investigated multimodal imaging features of both choroidal and sub-RPE non-reflective spaces together, hypothesizing that they were one entity, caverns. From donor eyes, we determined disease associations, prevalence, tissue localization, and morphology of globules in the maculas of AMD and age-matched control eyes. We find that the imaging characteristics and tissue localization of caverns can be explained well by histologic globules, which in turn appear to be part of normal chorioretinal physiology.

Methods

Compliance

This observational, consecutive case series was approved by the Western Institutional Review Board in New York and the Institutional Review Board at University of Milan. The

laboratory histopathology study was approved by the Institutional Review Board at University of Alabama at Birmingham. Research adhered to the Tenets of the Declaration of Helsinki and complied with the Health Insurance Portability and Accountability Act.

Histology

We utilized Project MACULA (<http://www.projectmacula>), a National Eye Institute- and foundation-funded online resource of human AMD histology.¹⁰ To create Project MACULA, high-resolution histology of 139 maculas was surveyed systematically and photo-documented. Eyes were accessioned for research purposes from non-diabetic white donors to the Alabama Eye Bank during the period 1996–2012. Median death-to-preservation time was 3:49 hours (range, 0:40–11:40 hours). Ophthalmic health records were not available for most donors. Eyes were preserved by immersion in 1% paraformaldehyde and 2.5% glutaraldehyde in 0.1M phosphate buffer following anterior segment excision. Although *ex vivo* color photography and SD-OCT was performed, caverns were not visible, likely due to incomplete light penetrance through edematous retina.

Tissue punches 8 mm in diameter and containing the fovea and the temporal portion of the optic nerve head were post-fixed by osmium tannic acid paraphenylenediamine (OTAP) to accentuate extracellular lipid. They were embedded in epoxy resin (PolyBed 812, Polysciences, Warrington PA) for 0.8 μ m-thick sections that were stained with 1% toluidine blue for polychromaticity.¹¹ At two levels (foveal and perifoveal 2 mm superior to the foveal center), sections were scanned with a 40X objective (numerical aperture = 0.95) for systematic review, annotation, and layer thickness measurements. One tissue block was re-sectioned horizontally to provide a view comparable to *en face* SD-OCT. Several eyes were sectioned at silver-gold thickness and viewed with a transmission electron microscope (1200 EXII, JEOL USA, Peabody, MA; AMTXR-40 camera, Advanced Microscopy Techniques, Danvers, MA).

As described,¹² AMD cases were defined via histopathology as the presence of one large druse (>125 μ m in diameter) in the macula or severe RPE changes in the setting of at least one druse or continuous basal linear deposit, with or without the presence of neovascularization and its sequelae. Eyes with geographic atrophy (GA) had at least one region 250 μ m in diameter lacking a continuous RPE layer (but possibly containing ‘dissociated’ RPE). Unremarkable eyes were those lacking characteristics of AMD or other chorioretinal disease as discernable in either histology or *ex vivo* imaging; these served as comparison eyes.

At each location in a standard grid overlaid on the foveal and perifoveal cross-sections, thicknesses were measured and morphologies indicated from a layer-specific drop-down menu with a field for free comments (‘M&A’ a custom ImageJ plug-in; <https://fiji.sc/>). Thus, the globule encounter rate is an unbiased estimate of tissue prevalence in these eyes. At the same time, sections were imaged with a light microscope (Nikon Eclipse, Melville NY), a 60X oil-immersion objective (numerical aperture = 1.4) and digital camera (XC10; Olympus, Center Valley PA). From these images, some of which were taken outside the systematic sampling locations, 117 globules were chosen for morphologic description and diameter measurements (ImageJ, <https://fiji.sc/>). In creating figures, digital light and electron

microscopic images were adjusted for exposure, contrast, sharpness, and white balance (Photoshop CS6, Adobe, San Jose, CA).

Clinical imaging

The clinical study was conducted between June 2016 and December 2016 at two different settings: Vitreous Retina Macula Consultants of New York, USA, and the Eye Clinic, Department of Biomedical and Clinical Sciences “Luigi Sacco” of Milan, Italy. Consecutive cases diagnosed with clear visualization of choroidal and sub-RPE caverns on *en face* and cross-sectional OCT scans were progressively included. Patients diagnosed with different retinal diseases and healthy controls undergoing OCT examination were included.

Clinical and demographic data including ocular diagnosis, age and laterality of the lesions (unilateral or bilateral) were recorded. To investigate possible associations with systemic conditions, the presence of atherosclerotic disease, hyperlipidemia, hypertension and diabetes mellitus were also recorded. All study eyes were evaluated with structural and angiographic OCT using the Spectralis HRA+OCT (Heidelberg Engineering, Heidelberg, Germany), Optovue RTVue XR Avanti (Optovue Inc, Fremont, CA, USA), PlexElite (Carl Zeiss Meditec, Inc., Dublin, California, USA), or Triton Plus DRI-OCT (Topcon Medical Inc., Oakland, NJ). Many eyes were also imaged with high-resolution digital color and red free (RF) fundus photography, fundus autofluorescence (FAF), fluorescein angiography (FA) and/or indocyanine green angiography (ICGA) using the Topcon TRC-50XF fundus camera (Topcon Medical Systems, Paramus, NJ, USA). In other cases, FAF images, FA, ICGA, near-infrared reflectance (IR) and confocal multispectral (MultiColor) imaging were obtained with the Spectralis HRA + OCT (Heidelberg Engineering, Heidelberg, Germany).

In this study, caverns were identified not only in the choroid of cases with geographic atrophy secondary to AMD as described previously,⁵ but also in cases with intact RPE. Thus, we also recorded the presence of sub-RPE caverns, defined as non-reflective spaces within sub-RPE neovascular tissue. In all locations, a characteristic tail of hypertransmission into the choroid was observed, which was illustrated in the original description⁵ without comment. The tail corresponded to an area of increased OCT signal transmission underlying the cavern, similar to the hypertransmission described in GA, due to the lack of shadowing from the RPE layer, but on a smaller scale.¹³

Caverns were first identified as non-reflective spaces in *en face* OCT reconstructions, and then confirmed with cross-sectional OCT scans, which also revealed the characteristic hypertransmission tail. Cross-sectional scans were also used to differentiate caverns from structures such as pigment, vessels, and refractile drusen,¹⁴ which also shadow, but lack tails of posterior hypertransmission. To obtain a range of sizes, the maximum diameter of smaller and larger lesions in each case was measured using the manual caliper in the OCT software.

Bibliographic search

To link the histologic and clinical imaging parts of the study and propose physical explanations for tissue optical phenomena, we drew inferences about globule composition and consulted literature reporting the OCT reflectivity of structures known to be lipid-rich.

Results

Histologic survey and description of globules

Demographics of 139 study eyes from 139 donors (89 females, 50 males, mean age 82.9 ± 8.4 years) that were reviewed by light microscopy are provided in Table 1. Of this total sample, 52 eyes had unremarkable maculas, 26 eyes had early AMD, 13 eyes had GA, and 40 eyes had neovascular AMD. We found that 61/139 eyes (43.9% of the total sample) exhibited globules. Of these 61 eyes, 22 (15.8% of the total sample) had unremarkable maculas, 8 (5.8%) had early AMD, 4 (2.9%) had geographic atrophy secondary to AMD, 21 (15.1%) had neovascular AMD, and 6 (4.3%) had other or unknown pathology. Globule distribution across diagnostic groups did not differ significantly.

A total of 144 globules encountered in the systematic survey provide a basis of tissue prevalence estimates. Globules were found primarily in the choroidal stroma (131, 91.0%) but also in the sclera (7, 4.9%), and within the stroma of subretinal and sub-RPE neovascular membranes (3, 2.1%). Several small profiles resembling globules were also found in capillaries (2 in choriocapillaris, 1 in retina, 2.1%). Of these globules, 117 were characterized in detail (Table 2).

Figure 1 and Supplementary Information-Figure 1 provide panoramic views of tissues containing globules. In an eye of a 69-year-old woman with an unremarkable macula, numerous globules were seen in the inner choroid (Sattler's layer) underlying the fovea and perifovea (Figure 1A, B), where they occupied more than half of choroidal thickness (Figure 1C). In an *en face* view of this donor eye (Supplementary Information-Figure 1), globules are shown singly and in groups of 2 to 7 among arterial cross-sections. Figure 1D shows an eye of a 90-year-old woman with disciform degeneration secondary to neovascular AMD. Remarkably, globules are found in a continuous line from the choroid, passing along the neovascular tissue stalk into the fibrovascular scar.

Figures 2–3 shows the morphology of individual globules, which in this preparation stain brown to olive-green. We observed solitary and well circumscribed globules (Figure 2A) and double or multilobular globules (Figure 2B, D), large enough to elevate Bruch's membrane (Figure 2B) and compress surrounding cells (Figure 2A, B). Globules were acellular and could lack internal features (as in Figure 1C) but most showed signs of activity, such as pitting (Figure 2C), dispersion (Figure 2E), disintegration (Figure 2F), or crystal formation (Figure 2G). Globules were situated among 'entombed' RPE within the neovascular tissue in the sub-RPE space (Figure 2H), and within the sclera, they were usually found near penetrating vessels (Figures 2G, 3A). Mean diameters of intact globules were 65.4 ± 27.9 μm (multilobular) and 58.9 ± 37.8 (solitary) (Table 2). Globules are as large as or larger than choroidal vessels, by inspection, and by comparison of measured diameters to reported luminal diameters in human choroid.¹⁵ We confirmed with electron microscopy that globules were extracellular, i.e., lacking an enclosing membrane (Figure 3B). In none of these investigated sites did we see evidence of any globule-associated, cell-driven inflammatory processes, i.e., infiltrated leukocytes or macrophages.

Before linking histology to imaging, we offer initial hypotheses about globule composition. We like Friedman demonstrated osmophilia,⁴ indicating saturated fats. Globules were shown by Friedman to bind Sudan IV, a lysochromic (fat-soluble) diazo dye used mainly for demonstrating triglycerides in frozen tissue sections. Interestingly, stained globules were not observed in studies using filipin histochemistry for esterified and non-esterified cholesterol in donor eyes.¹⁶ Globules were also not reported in studies using ultraviolet or blue-light elicited autofluorescence,^{16,17} suggesting that retinoids are a minor component. From these limited data, we conclude that the most likely major globule constituent is triglycerides, i.e., fatty acids esterified to glycerol, a trivalent alcohol.

Clinical imaging and description of caverns

A total of 41 eyes from 28 patients were included in the clinical imaging study. Of these, 16 eyes had non-neovascular AMD, 8 eyes had neovascular AMD, 6 eyes had pachychoroid spectrum of diseases, 4 eyes had Stargardt disease, 2 eyes had cone dystrophy, 1 eye had choroidal hemangioma, and 4 eyes were unremarkable and appeared healthy. Demographic and clinical data are summarized in Table 3. Patients presented with atherosclerotic disease and ischemic heart disease in 5 cases (17.9%), hyperlipidemia in 8 cases (28.6) and hypertension in 10 cases (35.7%). Also, 2 patients presented with diabetes mellitus (7.1%). Caverns were clearly visible on *en face* and cross-sectional OCT in 41 eyes (100%), by color fundus photography in three eyes (7.3%), by RF fundus photography in one eye (2.4%), and by IR in 17 eyes (41.5%). No caverns were initially identified by FAF, FA. Only one case showed late hyperfluorescence in the area of the caverns on ICGA. By OCT, the smallest caverns had a mean diameter of 44.8 μm [range: 15–97], whereas the largest caverns measured 105.9 μm in diameter [range: 34–232].

Patients showing atrophy of the RPE—Twenty eyes of 13 patients diagnosed with GA of the RPE due to non-neovascular AMD (Figure 4, 5) or Stargardt disease (Figure 6) demonstrated multiple choroidal caverns on *en face* OCT reconstructions within the atrophic area, and all were confirmed by cross-sectional OCT. Within these 20 eyes, caverns were also identified on color photography in 2 cases (10%), on RF and ICGA images in 1 case (5%), on IR images in 11 cases (55%). FAF and FA failed to show corresponding features in these areas. Cross-sectional OCT scans were particularly useful for differentiating caverns from refractile drusen (Figure 5, I-II). Both were reflective on projection color and confocal MultiColor imaging and hyperreflective on IR imaging. However, caverns had hypertransmission tails, whereas refractile drusen, being non-transmissive for light, cast shadows.

A case diagnosed with neovascular AMD complicated by an RPE tear (Supplementary Information-Figure 2) demonstrated caverns within the area of denuded RPE. Caverns identified on structural OCT scans lacked flow signal by OCT-A. Caverns were also observed on color fundus photography as bright round spots and on NIR as hyperreflective areas. FAF failed to show comparable features within the area of hypoautofluorescence associated with denuded RPE.

Patients showing intact RPE—Thirteen eyes of 9 patients had intact RPE in the settings of pachychoroid spectrum of diseases including central serous chorioretinopathy (Figure 7), cone dystrophy, choroidal hemangioma, and control healthy cases (Supplementary Information-Figure 3). Despite intact RPE, caverns could be most easily identified on *en face* OCT and confirmed with cross-sectional OCT scans, even though caverns were frequently solitary in these cases. Color photography, RF, FAF, FA, ICGA, and NIR failed to show changes in areas corresponding to the caverns.

Patients showing sub-RPE caverns—Seven eyes of 5 patients diagnosed with neovascular AMD (Figure 8) demonstrated caverns within the neovascular sub-RPE tissue, seen as multiple non-reflective, angular-to-round non-reflective spaces with the characteristic tail of hypertransmission on cross-sectional OCT scans. In *en face* OCT reconstructions, typical non-reflective spaces were observed in 6 cases (85%). On NIR images, caverns appeared hyperreflective in 6 cases (85%). Caverns were not identified on color photographs, RF, FAF, FA, or ICGA images.

Although caverns in clinical imaging are suggested as non-perfused vessels, we drew on globule composition and tissue distribution to check published OCT visualizations of lipid-rich structures. OCT reflectivity originates at boundaries between materials of different refractive index, and previous studies have documented OCT signatures of lipid-containing tissue features with high-refractive index (Supplementary Information, Table 1). These include adipocytes, necrotic core of atheroma, phagocytes containing lipid droplets, and silicon oil retained after surgical tamponade. In general, large structures (necrotic cores, ~2 mm extent; adipocyte lipid droplets; 100 μ m diameter) are hyporefective, and small structures (intracellular lipid droplets, 1–2 μ m diameter) are hyperreflective (Supplementary Information, Table 1). A spherical shape is energetically efficient for lipid in an aqueous environment, and spherical globules joined together (Figure 2B, G) could create an “angular” cavern. Focal internal reflective features may represent crystallizations (Figure 2G). An especially intriguing cavern characteristic is a prominent and consistent posterior tail of hypertransmission, which differentiates caverns from choroidal vessels, “round hyporefectivities” considered cavern precursors,⁸ and fluid-filled cysts or cavitations. Tails may relate to lower absorption of incoming light by lipid than by surrounding tissue (Supplementary Information, Table 2). In particular, blood (including oxygenated and deoxygenated hemoglobin) in choroidal vessels absorbs 10–1000 times more light than lipid over the spectral region 800 nm –850 nm. Thus, more light passes through a globule and into underlying choroidal stroma and sclera than through vessels, forming a hyperreflective tail. Consistent with this model, peripapillary intrachoroidal cavitations in myopia are agreed to be fluid-filled, and they lack a hypertransmission tail.^{18,19} Explaining some cavern optical attributes will need additional data, however. For example, posterior reflective boundaries were not shown in large lipid-containing structures in arterial intima (Supplementary Information, Table 1), possibly due to limitations of time-domain OCT and specimen size.

Discussion

Our histologic description of lipid globules is the most comprehensive since Friedman’s 1966 publication, and thus we re-launch inquiry into their biologic significance. Our study

adds to prior clinical literature on caverns by demonstrating these OCT features in healthy subjects and in conditions beyond GA secondary to AMD. (Table 3) Because we identified non-reflective features consistent with caverns within the sub-RPE neovascular tissue in cases of neovascular AMD, we dropped the descriptor ‘choroidal’ from the terminology. Using multimodal imaging, we recognized defining characteristics of caverns: 1) non-reflective spherical to polyhedral structures visible on *en face* and cross sectional OCT, as described;⁵ 2) Posterior tail of hypertransmission on cross-sectional OCT (B-scan), as illustrated;⁵ 3) In cases of RPE loss, frequently hyperreflective on NIR and rarely reflective on color photographs or hyperfluorescent on ICGA. 4) Not visible on FA or FAF; 5) No evidence of flow signal on *en face* or cross-sectional OCT-A. We did not find punctate or linear hyperreflectivities within the caverns, as described,⁵ because we selected cases with non-reflective spaces.

Histologic globules are common, appearing in 43.9% of donor eyes in a research archive. Because diabetic donors were excluded, we cannot evaluate Friedman’s statement that this patient population has a predilection for globules. We did confirm that globules are both extracellular and extravascular. Yet they clearly associate with vessels, not only in the choroid, but also with vessels penetrating the sclera, and also, strikingly, those in neovascular membranes. By prevalence and extravascular location, globules can be distinguished from intravascular fat emboli, which may contribute to Purtscher retinopathy, a rare occlusive condition associated with trauma.²⁰ Because of their small size, high apparent lipid content, and absence of cells, internal septa, and capsules, globules can be differentiated from lipomatous tumors, a heterogeneous group of benign and malignant soft tissue masses.^{21,22} There was no evidence of prior hemorrhage leading to extracellular cholesterol deposition, as occurs in atheroma.²³ Like Friedman, we observed an absence of cell-mediated inflammation in the stroma surrounding globules. Significantly, it appears that globules are dynamic, with a lifecycle, because our high-resolution histology evidenced pitting, crystallization, and dispersion, consistent with extracellular activities such as enzymatic remodeling. The compression of surrounding cells, although not universal, is inconsistent with extended chronicity. We cannot determine from our current data if globules formed in place or migrated from elsewhere. Once in place, however, they seem to be part of the choroidal ecosystem. These considerations strongly suggest that globules represent a heretofore-unappreciated aspect of normal chorioretinal physiology.

Caverns were detected using *en face* viewing of high-density OCT volumes as non-reflective areas, allowing us to exclude shadowing using high quality cross-sectional OCT on the corresponding hyporefective areas. In areas of RPE loss, color fundus examination was able to show a bright appearance only in 3 of 21 cases (14.3%), being easily misdiagnosed as refractile drusen. (Figure 5A) In these cases, cross-sectional OCT was again the most useful modality to differentiate these features. On the other hand, NIR images acquired with the Spectralis confocal SLO show hyperreflective, round features in some (55%) cases with RPE loss (Figure 5D; Figure 6B; Supplementary Information-Figure 2E), but not all (Figure 4B), due to limited depth resolution. A variable appearance on NIR reflectance may be attributable to the differences in location and relative size of these features. In the presence of a cavern occupying most of the choroidal thickness, we can see the reflectivity of the underlying sclera, whereas in the presence of relatively smaller caverns with underlying

preserved choroid, scleral reflectivity will be blocked as demonstrated directly in GA areas.²⁴ Similarly, only one cavern was visible on late ICG. Although we cannot explain this phenomenon as of yet, we cannot exclude the possibility that changes in the surrounding choroid (superior and inferior to the globule) contributed. In cases showing preserved RPE, neither color photography nor NIR imaging revealed any features corresponding with caverns. (Figure 7, A and B) In neovascular AMD, NIR imaging showed the presence of sub-RPE caverns in the majority of cases (85%); color fundus photography was not useful. (Figure 8) Other imaging modalities as FA, ICGA and FAF did not show evidence of caverns in any of our cases. (Figures 3, 5, 6, 8 and Supplementary Information-Figure 2) Finally, *en face* and cross-sectional OCT-A images confirmed no flow signal within these non-reflective areas. (Figure 8, F and H; Supplementary Information-Figure 2, G and I) We note that previous investigators saw ‘bubbles’ in choroids of choroideremia patients by OCT and adaptive optics SLO, speculating a correlation to ‘lipid droplets’ seen in histology of a choroideremia carrier;^{25–27} these studies did not reference Friedman’s description.

Our interpretation correlating globules and caverns is an alternate to the initial idea that caverns correspond to hypo-perfused or regressed vessels.⁵ In our study flow signal was absent from choroidal caverns and present in nearby vessels on OCT-A (Figures 6H, Supplementary Information-Figure 2I) although projection artifact could remain. However, all encountered caverns were spherical-to-polyhedral rather than tubular in shape, as expected for vessels, and they were located near or directly adjacent to choroidal vessels. Non-perfusion is best documented for choriocapillaris in histology and possibly also in OCT-A.²⁸ Choriocapillaris endothelium becomes less functional in aging as indicated by reduced immunoreactivity of CD34, a vascular marker.²⁹ In AMD, this endothelium retracts from Bruch’s membrane, involutes, and is cleared by phagocytes,³⁰ thus lowering choriocapillary apposition to Bruch’s membrane from ~70% to <50%.^{31,32} Non-perfused choriocapillaries are too small, located too far inward in the choroid, and too numerous to account for caverns. Regarding macro-vessels, the choroid thins with age³² in concert with defined molecular changes.²⁹ Whether choroidal vessels of the same diameter¹⁵ and location as caverns are non-perfused and contribute to overall thinning via regression, occlusion, hemostasis, or some combination, has not been directly investigated. However, if vessels were to involute, as previously described on AMD,³³ and control subjects with atherosclerosis and hypertension,^{34,35} one might expect histologically detectable occlusive fibro-intimal thickening with collagenous proliferation, and thus a mild hyperreflectivity on OCT images, rather than non-reflectivity, as observed for caverns.

The high prevalence of globules in the choroid, strong circumstantial evidence that they follow choroidal vessels into neovascular tissue, and evidence that they are exceedingly rare in neurosensory retina together suggest that globules serve a function specific to outer retinal cells served by the choroid. As reviewed,³⁶ animals and humans have a complex, finely tuned, and long-studied system for lipid nutrient delivery. At its center is the intravascular hydrolysis and release of fatty acids from triglycerides in plasma lipoproteins, by the enzyme lipoprotein lipase (LPL) in concert with others that transfer LPL across capillary endothelium from synthesis sites in parenchyma to activity sites in vascular lumens. As befitting the huge requirement of retinal membranes for Ω -3 fatty acids, expression of LPL

and other intravascular lipolysis genes is strong in vascularized tissues of human eyes, especially in the choroid and notably absent from non-vascularized outer retina^{36,37} (Supplementary Information, Table 3). Recently it was shown that photoreceptors depend on fatty acid β -oxidation for energy, challenging a long-held view that neurons consume only glucose.³⁸ These disparate data prompt our proposal that globules are mobilizable, intraocular storage sites for fatty acids of dietary origin required by photoreceptors for metabolism and membrane integrity. Neither caverns nor globules are specific to atrophic areas,⁵ or even to atrophic eyes, although RPE absence facilitates *in vivo* detection. Further, absence of photoreceptors may reduce the demand that drives lipolysis, if our theory is correct, thus explaining globule persistence in atrophy.

This study has limitations and offers directions for future study. The clinical imaging study had a relatively small sample, lacked comprehensive prevalence estimates, and lacked detailed quantitative analysis of cavern size. The histology study had unbalanced diagnostic groups in a convenience sample and lacked direct clinicopathologic correlation and extensive electron microscopic analysis. Nevertheless, by establishing multimodal imaging parameters for future studies, we also showed that in demographics, tissue localization, prevalence, size, and optical properties, caverns match well histologic globules. Although new data on tissue optics are welcome, it would be remarkable with this level of correspondence if globules were not the histological correlate of caverns, as it would imply that another choroidal feature this large and distinctive remains to be detected clinically. Our biologic hypothesis has many gaps to fill but if tested, can provide important insights into outer retinal biology important for AMD. The principal ultrastructural component of soft drusen, lipoproteins of RPE origin, is rich in the fatty acid linoleate, implicating diet as a source.³⁹ Therefore, a multi-disciplinary investigation of how fatty acids are delivered to and utilized in outer retina and how globules fit in should prove fruitful in elucidating new therapeutic strategies. Towards this end, globule dynamism studied through longitudinal clinical imaging, genetic associations of caverns, and globule composition should be future research goals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Panoramic view of Friedman lipid globules in normal choroid and in a neovascular membrane.

A, B, C. 69-year-old woman, unremarkable macula; **D.** 90-year-old woman, choroidal neovascularization, and fibrovascular scar. **A.** Lipid globules line up along the inner choroid of foveal section (fuchsia arrowheads). ONH, optic nerve head; R, retina; C, choroid; S, sclera. **B.** Lipid globules line up along the inner choroid of perifoveal section (fuchsia arrowheads). **C.** Globules (fuchsia arrowheads) occupy half or more of choroidal thickness. From left to right, globules are multilobular, solitary, multilobular, solitary. INL, inner

nuclear layer; ONL, outer nuclear layer; RPE, retinal pigment epithelium; Ch, choroid; S, sclera. **D.** Globules (fuchsia arrowheads) track from the choroid into neovascular membranes. A large intraretinal cyst with lipid-containing fluid and phagocytes is also present (green arrowhead). R, retina; FV, fibrovascular scar; C, choroid.

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Figure 2: Morphology of lipid globules.

Submicrometer epoxy resin sections of OTAP-post-fixed tissue, toluidine blue stain. **A.** Solitary and well circumscribed globule, in an 82-year-old man with neovascular AMD. RPE, retinal pigment epithelium. Scale bar in H applies to all panels. **B.** A large double globule elevates Bruch's membrane (red arrowheads) in a 75-year-old man with an unremarkable macula. **C.** Globule with pitting on its surface in a 71-year-old woman with an unremarkable macula. **D.** Multilobular globules form a chain in a fibrovascular scar just above Bruch's membrane in a 77-year-old woman with neovascular AMD. **E.** Dispersing

globules in an 87-year-old woman with neovascular AMD. Scar, fibrovascular scar. **F.** Disintegrating globules (yellow arrowhead) in an 89-year-old man with neovascular AMD. **G.** Crystalline changes in a globule located adjacent to blood vessel in the sclera in a 77-year-old woman with neovascular AMD. **H.** Dissolving globules within a disciform scar in an 87-year-old woman with neovascular AMD.

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Figure 3. Lipid globules are extracellular.

A. A solitary lipid globule with pitting on one aspect is located near scleral vessels, 3 mm nasal to the fovea in an 80-year-old woman with RPE detachment. The red arrowhead indicates the part of the globule surface examined by transmission electron microscopy. **B.** Electron micrograph of lipid globule surface indicated in panel A. Globule surface topology is not consistent with delimitation by a membrane. For comparison, a nuclear membrane is indicated (blue arrowhead).

Figure 4. Choroidal caverns in a case of geographic atrophy due to atrophic age-related macular degeneration.

A. *En face* structural optical coherence tomography (OCT) reconstruction at the level of the choroid showing hyporeflective round lesions corresponding with choroidal caverns (arrowheads). **B.** Infrared reflectance (IR) does not show changes within the area of the caverns. **C.** Fundus autofluorescence (FAF) shows an area of hypoautofluorescence. **D.** On indocyanine green angiography (ICGA) the caverns appear hyperfluorescent (arrowheads). **E.** Structural cross-sectional OCT scan at the corresponding area on panels A-D (black/

white line). Pink lines show the level of choroidal segmentation. Choroidal caverns (arrowheads) are seen on panel A and E as irregular non-reflective structures located next to choroidal vessels and associated with a marked posterior increase on OCT signal (choroidal hypertransmission).

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Figure 5. Choroidal caverns and refractile drusen in a case of geographic atrophy due to age-related macular degeneration.

A. Color fundus photograph. **B.** MultiColor reflectance. **C.** Fundus autofluorescence. **D.** Near infrared reflectance. **E.** En face structural optical coherence tomography (OCT) reconstruction at the level of the choroid at the corresponding area on panels A-D (white box). I-II. Structural cross-sectional OCT scans at the corresponding area on panel E (green lines). Choroidal caverns (black arrows) and refractile drusen (white arrows) are seen on panels A and B as highly refractile structures and on panel D as hyperreflective dots. On

panel E, choroidal caverns appear as well-defined, round, non-reflective features with sharp boundaries, whereas refractile drusen are represented by irregular mildly hyporeflective structures with ill-defined boundaries. On scan I, a choroidal cavern appears within the choroid as non-reflective spaces with a characteristic hyperreflective tail, whereas on scan II, refractile drusen are heterogeneous hyperreflective structures with marked posterior shadowing in the outer retina.

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Figure 6. Choroidal caverns in geographic atrophy secondary to Stargardt disease.

A. *En face* structural optical coherence tomography (OCT) reconstruction at the level of the choroid showing multiple round hyporeflective structures suggestive of choroidal caverns. One of the lesions is pointed (arrowhead). **B.** Infrared reflectance (IR) demonstrates the cavern to be hyperreflective (arrowhead). **C.** Fundus autofluorescence (FAF) showing a marked area of hypoautofluorescence. **D.** Indocyanine green angiography (ICGA) demonstrates hypofluorescence in the area of atrophy. **E.** Structural cross-sectional OCT scan at the corresponding area on panels A-D (black/white line). Horizontal red lines show the level of choroidal segmentation. Vertical red line shows the location of a choroidal cavern (arrowhead) that appears as a non-reflective structure associated with a posterior choroidal increase on OCT signal (choroidal hypertransmission). Choroidal caverns are in close relationship with choroidal vessels as seen in panel A.

Figure 7. Multimodal imaging of the left eye of a 68-year-old male diagnosed with pachychoroid neovascularopathy.

A. Color fundus photograph. **B.** Red free image. **C.** En face optical coherence tomography (OCT) reconstruction at the level of the choroid showing a non-reflective lesion superior and temporal to the macula (blue and pink lines). **D.** Magnification of the area on the black box on image D. **E.** Cross-sectional swept-source OCT scan at the corresponding area on panel E. Yellow lines show the area of segmentation for image C. **F.** Magnification of the area on the white box on image E. The non-reflective lesion appears irregular-shaped and associates

a tail of posterior choroidal hypertransmission (white arrowheads), whereas the retinal vessels demonstrate shadowing of the OCT signal (black arrowheads). The correspondence of vessels and caverns is made on panels D and F (white dashed lines)

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Figure 8. Multimodal imaging of the right eye of an 84-year-old woman diagnosed with neovascular age-related macular degeneration. **A**, Color fundus photograph. **B**, Fundus autofluorescence (FAF) image. **C**, Red-free (RF) image. **D**, Infrared reflectance (IR) image. **E**, En face structural OCT and **F**, en face OCT angiography (OCTA) reconstructions at the level of the choroid at the corresponding areas on A–D (white-dashed box). **G**, Structural cross-sectional OCT scan at the corresponding area on (E) (blue line). Red lines show the area of segmentation for (E–F). **H**, Cross-sectional OCTA image with flow signal overlay. The nonreflective structures are located

within the neovascular tissue, along vessels with the characteristic tail of hypertransmission (arrowhead), but now showing flow signal on OCTA.

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Table 1:

Demographics of histology study eyes

	All eyes					Eyes with lipid globules					
Diagnostic category	N	F	M	Age	SD	N	%	F	M	Age	SD
Unremarkable	52	29	23	79.7	9.5	22	15.8%	7	15	76.1	10.0
Early AMD	26	19	7	83.5	8.9	8	5.8%	3	5	77.1	9.0
Geographic atrophy	13	9	4	85.5	4.7	4	2.9%	3	1	82.8	4.8
Neovascular AMD	40	26	14	85.5	7.1	21	15.1%	13	8	85.2	7.0
Other/unknown	8	6	2	82.9	2.9	6	4.3%	3	4	80.6	5.4
Total	139	89	50	82.8	8.4	61	43.9%	29	32	77.4	9.4

All donors were non-diabetic and white.

Table 2:

Distribution and morphology of 117 characterized Friedman lipid globules

Diagnostic group	N	%	Location	N	%	Morphology	N	%	Mean diameter, μm	Standard deviation, μm
Unremarkable	31	26.5%	Choroid	82	70.1%	Multilobular	32	27.4%	65.4	27.9
Early AMD	12	10.3%	Sclera	15	12.8%	Solitary, well-circumscribed	26	22.2%	58.9	37.8
Geographic atrophy	13	11.1%	Neovascular tissue	17	14.5%	Dispersing	22	18.8%	47.8	22.2
Neovascular AMD	42	35.9%	Capillaries	3	2.6%	Dissolving	16	13.7%	64.2	19.9
Other/unknown	19	16.2%				Disintegrating	9	7.7%	92.9	22.6
						Crystalline	7	6.0%	73.6	41.8
						Scattered	5	4.3%	18.4	11.5
TOTAL	117	100.0%		117	100.0%		117	100.0%	61.1	31.5

Table 3:

Demographics of clinical study eyes

Demographic data: n, 28 patients	
Age; mean $\pm$ SD	67.75 $\pm$ 19
Laterality	
Unilateral; n (%)	15 (52)
Bilateral; n (%)	13 (48)
Diagnosis: n, 41 eyes	
Non-neovascular AMD; n (%)	16 (39)
Neovascular AMD; n (%)	8 (19)
Pachychoroid spectrum; n (%)	6 (15)
Stargardt disease; n (%)	4 (10)
Cone Dystrophy; n (%)	2 (5)
Choroidal Hemangioma; n (%)	1 (2)
Healthy; n (%)	4 (10)
Multimodal imaging available: n, 41 eyes	
Structural OCT; n (%)	41 (100)
OCT-A; n (%)	41 (100)
Color fundus; n (%)	19 (46)
RF; n (%)	23 (56)
FA; n (%)	13 (32)
ICGA; n (%)	9 (22)
FAF; n (%)	25 (61)

AMD: age-related macular degeneration, FA: fluorescein angiography; FAF: fundus autofluorescence, ICGA: indocyanine green angiography, OCT: optical coherence tomography, OCT-A: OCT angiography, RF: red free, SD: standard deviation.