Macular Inner Retinal Layer Thickness in Relation to Photopic and Mesopic Contrast Sensitivity in Healthy Young and Older Subjects

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Submitted: July 19, 2018
Accepted: October 18, 2018

Citation: Puell MC, Palomo-Álvarez C, Pérez-Carrasco MJ. Macular inner retinal layer thickness in relation to photopic and mesopic contrast sensitivity in healthy young and older subjects. *Invest Ophthalmol Vis Sci.* 2018;59:5487–5493. https://doi.org/10.1167/iovs.18-25334

Purpose. To examine relationships between the thicknesses of ganglion cell (GC)-related macular layers and central photopic or mesopic contrast sensitivity (CS) in healthy eyes.

Methods. Measurements were made in 38 young and 38 older healthy individuals. Total, inner, and outer retinal layer (IRL) thicknesses were measured in the macula region through spectral-domain optical coherence tomography (SD-OCT) across three subfields, or rings, centered at the fovea: central foveal, pericentral, and peripheral. Ganglion cell complex and circumpapillary retinal nerve fiber layer thicknesses were also measured. Low-spatial-frequency CS for gratings presented at the central 10° visual field were measured through computerized psychophysical tests under photopic and mesopic conditions. Relationships were examined by uni- and multivariate regression analysis.

Results. Peripheral IRL thickness emerged as the only independent predictor of photopic CS ($P = 0.001$) in the young group and of photopic ($P = 0.026$) and mesopic CS ($P = 0.001$) in the older group. The slopes of regression lines used to predict CS from peripheral IRL thickness were significantly different for pair-wise comparisons of both photopic CS and age group ($P = 0.0001$) and mesopic CS ($P = 0.0001$) and age group. These models explained 37% of the variability in photopic CS and 36% of the variability in mesopic CS.

Conclusions. Macular IRL thinning likely due to GC loss was related to reduced photopic and mesopic CS in older healthy eyes. In contrast, in the young eyes, a thicker macular IRL, possibly indicating transient gliosis, was associated with reduced CS.

Keywords: contrast sensitivity, retinal ganglion cells, optical coherence tomography, macular inner retinal layer thickness, mesopic vision, glial cells

Age-related changes in neural vision substrates may explain the decline produced over time in some visual functions and may increase an individual's susceptibility to degenerative disease such as glaucoma, for which age is a prominent risk factor.[1] Glaucoma is characterized by retinal ganglion cell (RGC) loss and its associated thinning of the retinal nerve fiber layer (RNFL) producing both peripheral and central vision loss.[2] During aging, the RGC axon itself is highly vulnerable but RGC bodies are subject to variable age-related loss.[3–6] Histologic studies have detected reduced age-related RGC density in the central human retina.[7–10] For example, it has been reported that GC density was reduced by 25% in the central retinal 11° in older adult donor eyes compared to young adult donor eyes.[8] Despite this, many postmortem specimens obtained from even the oldest donors have shown cell counts in the range of much younger donors, and vice versa. As there is high interindividual variability, the effects of aging on the underlying neural mechanisms of visual decline during aging are still not well understood.

Visual contrast mechanisms are mediated through retinal ganglion cells[11,12] and spatial contrast sensitivity (CS) is a critical aspect of vision that declines during normal aging.[13,14] Research has indicated that the optical characteristics of older eyes are largely responsible for CS deficits produced in older adults at high spatial frequencies in conditions of photopic light levels[13] (see Ref. 13 for review). In contrast, neural factors have been attributed a greater role in explaining age-related reductions both in CS at low luminance or mesopic light levels[4,13] and in photopic low-spatial-frequency CS (below 2 cycles per degree [cpd]).[15] Aging and glaucoma especially lead to impaired low-spatial-frequency CS, which is presumably mediated by RGC with larger receptive fields.[16] Furthermore, the decrease produced in ganglion cell density during aging[8,9] is a logical candidate mechanism to explain decreased spatial CS under low luminance conditions in older adults.[17–19] However, this issue remains to be clarified.

In an effort to gain insight into the possible neuronal mechanisms underlying age-related visual function loss, this study examines the relationship existing between spatial CS and in vivo measures of RGC-related layer thickness (e.g., inner retinal layer, ganglion cell complex, circumpapillary RNFL) made on the healthy human macula through spectral-domain optical coherence tomography (SD-OCT). In patients with glaucoma, numerous studies have addressed structure-function relationships between visual field sensitivity and RNFL thickness changes produced in the optic nerve head (for review, see Ref. 2). These studies have confirmed that RGC loss leads to reduced light sensitivity.[20] Recently, the relationship between macular ganglion cell/inner plexiform (GC/IPL) layer thickness and photopic CS has been assessed in patients with glaucoma[21]

and also in a small sample of glaucomatous, aged or young eyes, all together.[22] However, the relationship between macular RGC-related layer thickness and photopic CS has been scarcely addressed in healthy subjects. Moreover, to the best of our knowledge, no study has tried to quantitatively relate macular thickness as revealed by SD-OCT to mesopic CS in healthy adults.

Visual ability in low illumination conditions and the capacity to detect low-contrast objects are two important functions in the daily life of older subjects. A common visual complaint in older adults despite having good visual acuity and good eye health is difficulty seeing under poor illumination conditions such as night driving or driving in fog or heavy rain.[13] Also, impaired vision in the dark or glare from bright lights are frequent complaints in individuals with glaucoma.[23–26] It is thus important to try to find some of the causes of such visual dysfunctions.

This study was designed to examine relationships between the structural factors macular inner retinal layer (IRL), ganglion cell complex (GCC), and circumpapillary RNFL (cpRNFL) thicknesses, and central low-spatial-frequency CS measured under photopic and mesopic luminance conditions in younger and older healthy eyes. This information will improve our understanding of how age-related changes in RGC-related macular layer thickness impacts spatial CS in the healthy aged eye compared to the younger eye. Our findings could also help identify structural and functional markers that may be useful for the early detection of subjects at a high risk of developing glaucoma so that suitable follow-up and prevention measures can be implemented.

Materials and Methods

Subjects

This cross-sectional study was conducted at the Faculty of Optics and Optometry, Universidad Complutense de Madrid, Spain. Subjects for the younger group ($n = 38$) were recruited among the university students. Subjects for the older group ($n = 38$) were recruited among students of the older adult studies program (Universidad para los Mayores, UCM). The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by our institution's review board. Subjects were informed about the study protocol before giving their written consent to participate.

In each healthy subject, measurements were made only in the eye showing best visual acuity (VA). If both eyes had the same VA, the right eye was selected. All participants were subjected to a careful ophthalmologic examination including measurement of VA and subjective refraction, a slit-lamp biomicroscopy of the anterior segment, intraocular pressure measurement and fundus examination performed at the University Optometry Clinic. Normal macula health was documented by color fundus photography and subsequent SD-OCT assessment. Subjects were required to have a best corrected visual acuity (BCVA) of 20/20 or better measured using the Snellen acuity chart in at least one eye, a refractive error no greater than ± 3.50 diopters (D) of sphere or ± 1.50 D of cylinder, and normal findings in the ophthalmologic examination. While we did not measure color vision, subjects reported having normal color vision. Exclusion criteria were: systemic disease such as diabetes, posterior subcapsular cataract; cortical or nuclear opacities greater than LOCS III classification grade 2; age-related macular degeneration; diabetic retinopathy; glaucoma; amblyopia; retinal vascular disease; or any other retinal abnormality. Subjects were also excluded if they were aphakic or pseudophakic or had undergone retinal surgery.

Spectral Domain Optical Coherence Tomography

Retinal thickness was measured by spectral-domain optical coherence tomography (SD-OCT) in one eye using an OCT system (3.3 iVue; Optovue Inc., Freemont, CA, USA). This instrument has been used to measure retinal thickness in individuals with glaucoma[27,28] and found to show excellent reproducibility.[29] The instrument acquires 26,000 axial scans per second resulting in an imaged area of approximately 6×6 mm centered at the fovea and has a depth resolution of 5 μm. Data were obtained using the Retina Map Scan protocol for macular thicknesses and the Glaucoma Scan protocol for GCC and RNFL thickness. Scans were captured through undilated pupils under dark room illumination and only high-quality images showing a Scan Quality Index >65 were included.

Macular thickness measurements were segmented automatically into three layers (total, inner, and outer retina) across the nine subfields of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid covering 5×5 mm. The whole retinal layer was measured from the internal limiting membrane to the retinal pigment epithelium, the inner retinal layer (IRL) from the internal limiting membrane to the outer limit of the inner plexiform layer (this IRL layer includes the mRNFL, GC layer, and IPL), and outer retinal layer (ORL) from the outer limit of the IPL to the retinal pigment epithelium. For each retinal thickness segmented, three subfields consisting of concentric rings were defined[30] using the ETDRS grid: the fovea, or central circle, with a diameter of 1 mm, the pericentral ring of inner diameter 1 mm and outer diameter 3 mm, and the peripheral ring of inner diameter 3 mm and outer diameter 5 mm. Thickness measurements in the pericentral and peripheral rings were estimated by averaging measurements made in the quadrants nasal, temporal, superior and inferior. Thus, mean macular thicknesses were calculated for each of the three retinal segmentations (total, IRL, and ORL) for the pericentral ring and peripheral ring. Because the inner retinal layer is nearly absent in the fovea, only total thickness was analyzed in this 1-mm diameter area in the center of the fovea.

The GCC scan protocol consisted of mapping 7 mm centered 1 mm temporarily to the fovea. The GCC layer comprised the RNFL, GC and inner plexiform layers. Circumpapillary RNFL thickness was measured by calculating data along a circle 3.45 mm in diameter around the optic disc. Only overall means of cpRNFL thickness values provided by the Scan Glaucoma protocol were entered in the analyses.

Contrast Sensitivity

Spatial achromatic contrast sensitivity was assessed using psychophysical software (Visual Psychophysics Engine, Cambridge Research Systems, Kent, UK). Vertical sinusoidal gratings of 1 cpd (cycles per degree) temporally modulated at 2 Hz were generated and displayed via a visual stimulus generator (ViSaGe; Cambridge Research Systems) at the center of a calibrated, gamma-corrected high-resolution CRT monitor (Mitsubishi 2070SB, Tokyo, Japan) with a mean background luminance of 10 cd/m^2 under dark-room illumination. The screen was calibrated automatically using a photometer (Colorcal; CRS Ltd, Cambridge, UK). Stimuli were presented at the central 10° of visual field at a viewing distance of 1 m.

Subjects were tested at photopic (10 cd/m^2) and mesopic (1 cd/m^2) background luminance. The level of 10 cd/m^2 was chosen because this is the most common perimetry background photopic light level; 1 cd/m^2 was chosen to minimize

the higher variability in CS produced when low light levels are used.[31] Mean screen luminance was reduced from 10 cd/m^2 to 1 cd/m^2 using one neutral density filter (type 211, 0.9 log units [LEE Filters Worldwide, Andover, Hampshire, UK]). Although a 10 cd/m^2 light source viewed through a 0.9 log units filter gives a theoretical luminance of 1.26 cd/m^2, the filter is not totally flat and the luminance measured with a light meter (MAVO-SPOT 2 USB light meter; Gossen Lighting Control, Nuremberg, Germany) was 1 cd/m^2.

To measure CS in each participant, we used a two-interval forced-choice (2IFC) grating detection task. In each test, the stimulus appeared randomly presented in one of two successive intervals, each lasting 340 ms. The two intervals, one with a grating and the other blank, were separated by 1500 ms, and a brief tone signaled each interval's onset. Subjects were instructed to report in which interval they saw the stimulus. This response was entered into the computer program by the observer.

An adaptive staircase threshold strategy was used to vary the Michelson contrast ([maximum luminance − minimum luminance]/[maximum luminance + minimum luminance]) of the stimulus and obtain the psychophysical thresholds. Starting contrast percentages were 5% and 10% for photopic and mesopic conditions, respectively.[19] At the beginning of the staircase procedure, for every two correct responses, contrast was reduced by 0.1 log units (2 dB), and for every incorrect response, contrast was increased by 0.2 log units (4 dB). After three reversals, the staircase threshold strategy changed, and contrast was reduced by 0.05 log units (1 dB) after two consecutive correct responses and increased by 0.1 log units (2 dB) after a single incorrect response. The procedure ended after another three reversals. Thresholds were recorded as the mean of the last three contrasts seen in log units.

Before data collection, all subjects were allowed practice runs. A chin and forehead rest was used to view the screen monocularly while wearing best spectacle correction if needed. The eye not being tested was covered. The test was conducted in a dark room. Participants were tested at the mesopic luminance level first, followed by photopic. A minimum of 10 minutes of adaptation time was provided for the mesopic luminance test. The subject was then allowed 5 minutes of adaptation time before the photopic version of the test. In addition, pupil size was measured using a Colvard infrared pupillometer (Oasis Medical, Glendora, CA, USA) at both the photopic and mesopic light levels.

BCVA was also measured monocularly using high-contrast logMAR letter charts at 100 cd/m^2. Values were expressed as the logarithm of the minimum angle of resolution (logMAR).

Statistical Analysis

All statistical tests were performed using a software package (Statgraphics Centurion XVI; Statpoint Technologies, Inc., The Plains, VA, USA). According to prior sample size for power calculations to detect statistical significance for an anticipated correlation coefficient of 0.50, an alpha risk of 5.0% and a power of 90% the minimum sample size estimated was 38 subjects per age group. The normality of data was checked using the Shapiro-Wilk test.

Data were compared between the younger and older age groups using the Student's *t*-test and Bonferroni's multiple testing correction for multiple comparisons. The Wilcoxon signed-rank test (W) was used as an alternative for outcomes that were not normally distributed. The Spearman correlation coefficient, which does not assume linearity of relationships and is relatively unaffected by outliers, was calculated to determine the association between structural (each retinal thickness measurement) and CS variables in each age group.

Table 1. Demographic and Baseline Characteristics of the Study Participants*

Characteristics	Young Age Group	Older Age Group	*P* Value
Eyes, *n*	38	38	
Age, y	22.4 ± 2.7 (19.0, 31.0)	62.0 ± 3.5 (54.0, 68.0)	0.0008
Sex, male/female	8/30	9/29	0.9999
BCVA, logMAR	−0.10 ± 0.06 (−0.26, 0.06)	−0.02 ± 0.07 (−0.14, 0.2)	0.0008
Spherical equivalent, D	−1.16 ± 1.61 (−4.13, +2.88)	0.60 ± 1.12 (−2.50, +2.75)	0.0008
Photopic pupil size, mm	3.87 ± 0.64 (2.00, 5.00)	2.99 ± 0.36 (2.00, 4.00)	0.0008
Mesopic pupil size, mm	5.95 ± 0.76 (4.00, 7.50)	5.32 ± 0.56 (4.00, 6.50)	0.0008
Photopic CS, log units	2.03 ± 0.12 (1.70, 2.30)	1.89 ± 0.15 (1.60, 2.20)	0.0008
Mesopic CS, log units	1.81 ± 0.14 (1.45, 2.10)	1.69 ± 0.14 (1.35, 1.90)	0.0016

BCVA, best corrected visual acuity measured using logMAR letter charts.

* Mean ± SD (min, max).

Then, through forward stepwise multiple linear regression, we determined the independent factors that were most strongly correlated with the dependent CS variables in each age group separately. To control for optical and morphologic factors, each retinal thickness measurement, age, BCVA, spherical equivalent (SE) and pupil size (to control for retinal illuminance), were entered as independent predictors in the model while each CS variable served as the dependent variable. Further, an analysis of covariance was performed to compare regressions of the effects of thickness and age group on the CS variables.

Results

The demographic and baseline characteristics of the subjects in the young and older groups are provided in Table 1. Spearman correlation coefficients for the relationship between contrast sensitivity measured under photopic and mesopic conditions and retinal thickness are shown in Table 2 for each age group. In the younger subjects, photopic CS showed significant correlation with macular IRL in the peripheral ring (rho = –0.40; $P = 0.0152$), GCC (rho = –0.42; p = 0.0099) and with cpRNFL thickness (rho = –0.36; $P = 0.0275$). These correlations were negative, meaning that an increased retinal thickness was correlated with a worse CS. In this young age group, there were no significant correlations between mesopic CS and retinal thicknesses. In the older age group, photopic CS showed significant positive correlation with macular IRL thickness in the peripheral ring (rho = +0.34; $P = 0.039$). Also, significant correlations were detected between mesopic CS and peripheral IRL (rho = +0.51; $P = 0.0021$) or GCC (rho = +0.33; $P = 0.0425$) thicknesses in the older group. As these correlations were positive, a reduced retinal thickness was correlated with a worse CS in this older age group.

For each age group, multiple linear regression analyses were conducted in which the dependent variables were each of the CS tested and the independent variables, or predictors, were age, retinal thicknesses, BCVA, SE and pupil size. In the older age group, a studentized residual that exceeded 3 was deleted from the analysis. The results of the models with significant outputs are summarized in Table 3, where beta coefficients represent the change in CS per each micron change in macular

Table 2. Spearman Rank Correlation Between CS Measured Under Photopic and Mesopic Conditions

Retinal Thickness Measurement	Young Age Group Contrast Sensitivity		Older Age Group Contrast Sensitivity	
	Photopic	Mesopic	Photopic	Mesopic
Total central fovea	−0.23 (0.158)	−0.10 (0.546)	−0.09 (0.588)	−0.05 (0.771)
Pericentral ring				
Total	−0.26 (0.108)	−0.16 (0.335)	0.02 (0.904)	0.11 (0.490)
IRL	−0.24 (0.143)	−0.32 (0.052)	0.26 (0.108)	0.26 (0.110)
ORL	−0.19 (0.250)	−0.05 (0.678)	−0.17 (0.299)	0.00 (0.997)
Peripheral ring				
Total	−0.28 (0.093)	−0.20 (0.215)	0.01 (0.957)	0.20 (0.214)
IRL	**−0.40** (0.250)	−0.29 (0.082)	**0.34** (0.039)	**0.51** (0.002)
ORL	−0.20 (0.226)	−0.07 (0.751)	−0.20 (0.236)	0.01 (0.945)
GCC	**−0.42** (0.010)	−0.24 (0.142)	0.03 (0.863)	**0.33** (0.043)
cpRNFL	**−0.36** (0.028)	−0.22 (0.188)	0.11 (0.487)	0.17 (0.292)

Retinal thickness measurements made in the study groups; *P* values are shown in parentheses. Significant rho coefficients are shown in bold.

IRL thickness. There was only one significant independent predictor of the linear relationships. Peripheral IRL thickness emerged as the only independent contributor to photopic CS ($r = -0.53$, $P = 0.0006$) in the younger group (Fig. A) and also to the photopic CS ($r = 0.37$, $P = 0.0257$) and mesopic CS ($r = 0.58$, $P = 0.0001$) in the older subjects (Fig. B). Statistical powers (alpha risk of 0.050) for the Pearson correlations were 94% in the younger age group, and 61% and 97% in the older age group for the photopic and mesopic conditions, respectively. In the younger age group, the estimated effect of a 10-µm increase in IRL thickness on photopic CS was a 0.11 log-unit decrease. In the older age group, the estimated effects of a 10-µm decrease in IRL thickness on photopic and mesopic CS were reductions of 0.10 and 0.15 log units respectively. The other retinal thickness measures, age, BCVA, SE or pupil size variables were not found to be significant predictors of CS variables in each age group. Furthermore, no significant difference in mean peripheral IRL thickness was found between the young and older group.

We also compared regression lines to predict CS including the interaction term age group × peripheral IRL thickness. A significant difference emerged between regression line slopes (estimated interaction = 0.0218, $P = 0.0001$) for photopic CS. Also, significant differences were detected between the slopes (estimated interaction = 0.0224, $P = 0.0001$) of regression lines for mesopic CS. These models explained 36.8% of the variability in photopic CS and 35.8% of the variability in mesopic CS.

To further analyze which quadrant (nasal, temporal, superior and inferior) or mean thickness of the IRL in the peripheral ring was contributing more to CS, multiple linear regression analyses were conducted. In the younger group, mean peripheral IRL thickness was again the only contributor to photopic CS ($P = 0.0006$). In the older age group, the nasal quadrant of the peripheral IRL emerged as the only independent contributor to both photopic CS ($P = 0.0125$) and mesopic CS ($P = 0.0006$).

Discussion

This study was designed to explore structure–function relationships in eyes showing normal macular health and good VA to gain insight into the neuronal mechanisms underlying age-related CS loss. Its main finding was that macular IRL thickness, as measured through OCT, was an independent predictor of low-spatial CS measured in the central 10° of the visual field in both the older and younger eyes. While a thinner macular IRL was related to worse CS in older eyes, a greater IRL thickness was related to worse CS in young eyes.

The relationship between macular thickness and CS has been scarcely examined in healthy and glaucomatous eyes.[21,22] In two recent studies, significant positive correlation was found between photopic CS and total central macular thickness in glaucomatous eyes[21] and between letter-recognition contrast thresholds at photopic light levels and macular ganglion cell/inner plexiform layer thicknesses in glaucomatous and aged and young healthy eyes.[22] In this last study, only 14 older and 13 young healthy subjects were included and in the former two studies, optical factors such as lens opacity, which determine higher contrast requirements were not measured. In our older age group, photopic CS showed significant Spearman correlation with IRL thickness in the peripheral macular ring and mesopic CS showed significant correlation with peripheral IRL and also GCC thicknesses. Both photopic and mesopic CS were found to be predicted by only one contributing variable, peripheral IRL thickness. Further, the estimated effect of a 10-micron reduction in this thickness was a decrease in photopic and mesopic CS of 0.10 log units and 0.15 log units respectively (Table 3; Fig.). Moreover, in a subsequent multiple regression analysis we found that IRL thickness in the nasal quadrant of the peripheral macular ring was the only independent contributor to both photopic and mesopic CS. This finding is supported by the results of a study by Curcio and Drucker,[8] who found diminished RGC density in the central 11° of vision in a wedge of nasal retina in healthy older adults compared to younger adults. The thinning observed here in the IRL associated with a worse CS in some of our older eyes could therefore reflect a loss of RGC and/or significant shrinkage of dendritic structures and cell bodies of

Table 3. Multiple Regression Models Yielding Significant Results Showing the Predictor Variable in the Two Study Groups

Dependent Variable Contrast Sensitivity	Predictor Thicknesses	β Coefficient	Model R^2	*F*	*P* Value
Younger age group					
Photopic CS	IRL peripheral	−0.0112	28.1%	14.07	0.0006
Older age group					
Photopic CS	IRL peripheral	0.0106	13.4%	5.43	0.0257
Mesopic CS	IRL peripheral	0.0147	34.2%	18.16	0.0001

β, standardized beta estimate.

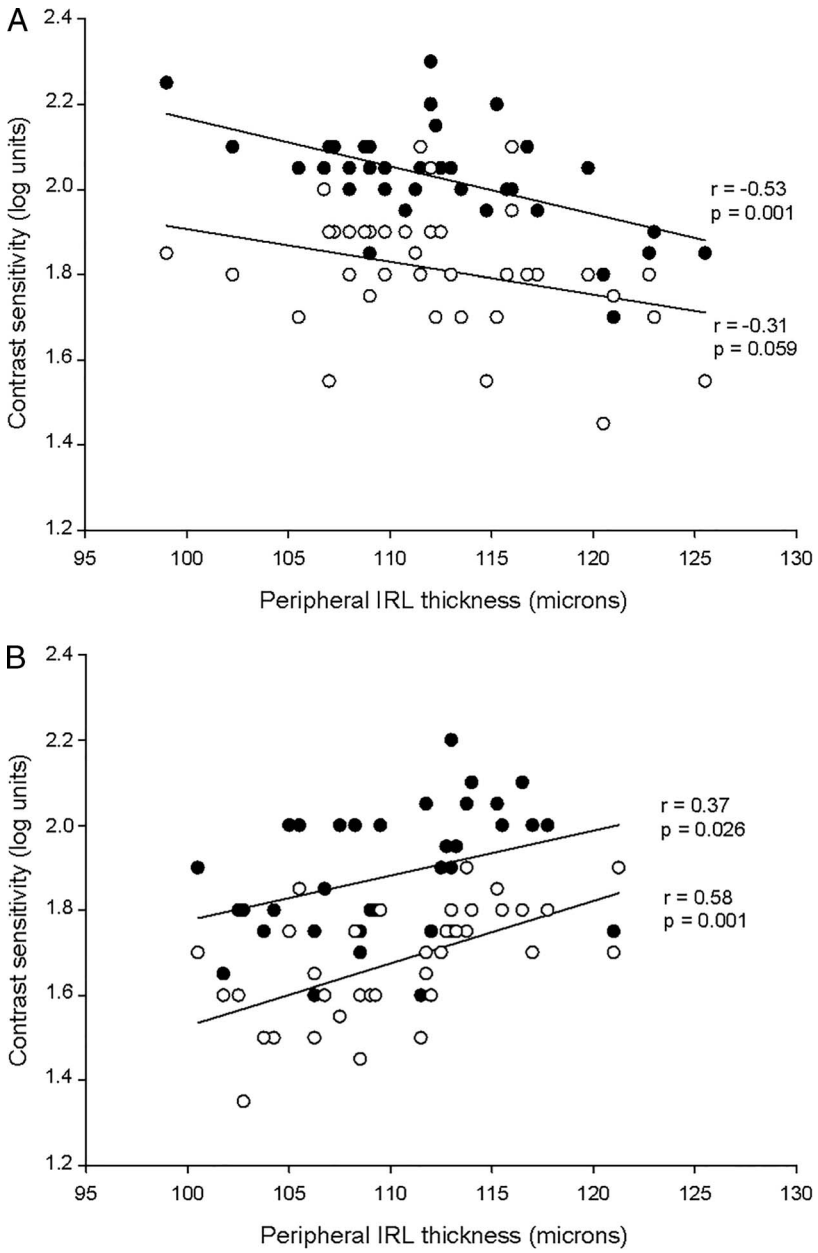

FIGURE. Correlation between IRL thickness in the macular peripheral ring and CS. (**A**) Photopic (*solid circles*) and mesopic (*open circles*) CS in the young age group, and (**B**) photopic (*solid circles*) and mesopic (*open circles*) CS in the older age group. *r*, Pearson's correlation coefficient.

the remaining cells. It should be noted that although mean peripheral IRL thickness was slightly thinner in the older group the difference was not significant ($P = 0.074$) when compared with the young age group. Studies examining the effect of age on the thickness of specific segments or layers of the macular region are scarce and results have been inconsistent. Thus, while one study found that GCL in the peripheral ring was not correlated with age,[30] another study showed that the thickness of the GC layer decreased with increasing age.[32]

It seems likely that the visual function loss that occurs in normal aging bears mechanistic similarities to that produced in glaucoma.[3] In this regard, in glaucomatous eyes with the earliest detectable visual field loss on automated perimetry, estimated RGC counts were reduced.[20] In our study, peripheral IRL thickness was able to explain up to 34% of the variance in mesopic CS but only 13% in photopic CS, likely reflecting a greater effect of the loss of RGC on mesopic than photopic function. Also, an alternative hypothesis is that the mesopic test places subjects at an adaptational disadvantage. In parallel, it has been reported that the low-luminance contrast multifocal visual-evoked potential stimulus is more sensitive than the conventional high-luminance contrast (black-and-white) stimulus in identifying early glaucoma.[33] According to Harwerth et al.,[5] it could be that a proportion of total circumpapillary RNFL thickness derived from non-neuronal (glial cells) tissue increases with age and thus partially compensates for an age-dependent loss of neuronal tissue. We would argue that something similar could occur in the IRL. Thus, if we were to correct for the nonneuronal component of IRL thickness, a stronger relationship between CS and macular OCT measurements would perhaps be found. In older individuals and glaucoma patients, hyperreflective structures (activated retinal astrocytes and Müller cells) seen on OCT en face imaging (cpRNFL) are common, whereas these structures are scarce in

younger individuals.[34] Also, in primary open-angle glaucoma, these hyperreflective structures have been more frequently identified in eyes showing lower retinal sensitivity and cpRNFL thickness.[35]

In our younger subjects, photopic CS showed significant correlation with the thickness measurements of peripheral macular IRL, GCC and cpRNFL (Table 2). Inner retinal layer thickness (the same substructure mentioned for the older age group), was the only independent contributor to CS measured in the central 10° of the visual field (Table 3; Fig.) and was able to explain up to 28% of the variance in photopic CS but not in mesopic CS. It is difficult to find an explanation for this because the variability in CS was similar under mesopic and photopic conditions and a loss of GCs in young subjects is unlikely. Contrary to the positive association found for the older group, correlation between IRL thickness and CS was negative for this younger group, meaning that the thickening noted gave rise to worse CS function. Although correlation between mesopic CS and peripheral IRL thickness in the younger eyes was not significant, when the regression lines of the two age groups were compared the interaction term was significant ($P = 0.0001$). Relationships between both photopic and mesopic CS and peripheral IRL were significantly different for each age group and the model was able to explain 37% and 36% of the variability in photopic and mesopic CS, respectively. The IRL thickening noted in some, supposedly healthy, young eyes could perhaps be attributed to increased glial tissue. Astroglial cells are strategically located to sense hypoxia in the inner layers of retina.[36] They defend the retina from damage through a process called reactive gliosis designed to maintain retinal homeostasis.[36] In mild to moderate forms of gliosis, cells may undergo hypertrophy (larger cell body size or thickening) and the enlargement of processes, yet if the trigger is removed, the cells could revert back to their former condition without altering the tissue.[37] The molecular changes that take place in the adult retina induced by damage occur rapidly, within minutes or hours.[36] In young healthy retinas, stress can be produced by external or internal factors such as smoking, dietary fat, or exposure to blue light[38] (e.g., emitted by electronic displays). Therefore, in some of our young subjects we propose that transient thickening of the macular IRL may have been responsible for the reduction observed in CS. In contrast, cpRNFL thickness could not be associated here with CS. This confirms the results of the single study conducted in healthy young subjects[39] and suggests that macular IRL thickness measures are better predictors of visual function than cpRNFL in healthy subjects. It should be noted that in our study, macular IRL and cpRNFL thicknesses were only moderately related (rho = 0.39, $P = 0.0166$) in young subjects.

The limitations of this study include its cross-sectional design. Further, it is possible that other methods of retinal segmentation able to measure individual retinal layers could yield more predictive models. An interesting question for future research arising from the findings of our study is to what extent central CS and its association with macular RGC layer thicknesses could serve to identify subjects at a high risk for later developing glaucoma.

In conclusion, increased macular IRL thickness was correlated with worse photopic CS in healthy younger eyes suggesting the involvement of glial cell changes in the retina. Macular IRL thinning, likely due to macular GC loss, was associated with reduced photopic and mesopic CS in older healthy eyes. Bearing in mind the large individual-to-individual variability in the effects of aging, measuring central CS in combination with macular RGC layer thickness through SD-OCT imaging could help identify the earliest stages of GC loss affecting visual function. Accordingly, this structure-function relationship may be a useful marker for the early detection of glaucoma.

Acknowledgments

The authors thank María Ayala-Ayerbes, MD, Jorge Chozas-Enrique, MD, and Sergio Gomez-Garcia, MD, for their help with subject recruitment and clinical measurements.

Supported by Santander-Universidad Complutense de Madrid Grant PR26/16-20275.

Disclosure: **M.C. Puell**, None; **C. Palomo-Álvarez**, None; **M.J. Pérez-Carrasco**, None

References

1. Coleman AL, Miglior S. Risk factors for glaucoma onset and progression. *Surv Ophthalmol*. 2008;53(suppl 1):S3–S10.
2. Yohannan J, Boland MV. The evolving role of the relationship between optic nerve structure and function in glaucoma. *Ophthalmology*. 2017;124:S66–S70.
3. Calkins DJ. Age-related changes in the visual pathways: blame it on the axon. *Invest Ophthalmol Vis Sci*. 2013;54:ORSF37–ORSF41.
4. Spear PD. Neural bases of visual deficits during aging. *Vision Res*. 1993;33:2589–609.
5. Harwerth RS, Wheat JL, Rangaswamy NV. Age-related losses of retinal ganglion cells and axons. *Invest Ophthalmol Vis Sci*. 2008;49:4437–4443.
6. Patel NB, Lim M, Gajjar A, Evans KB, Harwerth RS. Age-associated changes in the retinal nerve fiber layer and optic nerve head. *Invest Ophthalmol Vis Sci*. 2014;55:5134–5143.
7. Harman A, Abrahams B, Moore S, Hoskins R. Neuronal density in the human retinal ganglion cell layer from 16-77 years. *Anat Rec*. 2000;260:124–131.
8. Curcio CA, Drucker DN. Retinal ganglion cells in Alzheimer's disease and aging. *Ann Neurol*. 1993;33:248–257.
9. Gao H, Hollyfield JG. Aging of the human retina. Differential loss of neurons and retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci*. 1992;33:1–17.
10. Blanks JC, Torigoe Y, Hinton DR, Blanks RH. Retinal pathology in Alzheimer's disease. I. Ganglion cell loss in foveal/parafoveal retina. *Neurobiol Aging*. 1996;17:377–384.
11. Enroth-Cugell C, Robson JG. The contrast sensitivity of retinal ganglion cells of the cat. *J Physiol*. 1966;187:517–552.
12. Lee BB. Visual pathways and psychophysical channels in the primate. *J Physiol*. 2011;589:41–47.
13. Owsley C. Aging and vision. *Vision Res*. 2011;51:1610–1622.
14. Sia DIT, Martin S, Wittert G, Casson RJ. Age-related change in contrast sensitivity among Australian male adults: Florey Adult Male Ageing Study. *Acta Ophthalmol*. 2013;91:312–317.
15. Elliott D, Whitaker D, MacVeigh D. Neural contribution to spatiotemporal contrast sensitivity decline in healthy ageing eyes. *Vision Res*. 1990;30:541–547.
16. McKendrick AM, Sampson GP, Walland MJ, Badcock DR. Contrast sensitivity changes due to glaucoma and normal aging: low-spatial-frequency losses in both magnocellular and parvocellular pathways. *Invest Ophthalmol Vis Sci*. 2007;48:2115–2122.
17. Puell MC, Palomo C, Sánchez-Ramos C, Villena C. Normal values for photopic and mesopic letter contrast sensitivity. *J Refract Surg*. 2004;20:484–8.
18. Schefrin BE, Tregear SJ, Harvey LO, Werner JS. Senescent changes in scotopic contrast sensitivity. *Vision Res*. 1999;39:3728–3736.
19. Gillespie-Gallery H, Konstantakopoulou E, Harlow JA, Barbur JL. Capturing age-related changes in functional contrast

sensitivity with decreasing light levels in monocular and binocular vision. *Invest Ophthalmol Vis Sci*. 2013;54:6093–6103.

20. Medeiros FA, Lisboa R, Weinreb RN, Liebmann JM, Girkin C, Zangwill LM. Retinal ganglion cell count estimates associated with early development of visual field defects in glaucoma. *Ophthalmology*. 2013;120:736–744.
21. Fatehi N, Nowroozizadeh S, Henry S, Coleman AL, Caprioli J, Nouri-Mahdavi K. Association of structural and functional measures with contrast sensitivity in glaucoma. *Am J Ophthalmol*. 2017;178:129–139.
22. Chien L, Liu R, Girkin C, Kwon M. Higher contrast requirement for letter recognition and macular RGC+ layer thinning in glaucoma patients and older adults. *Invest Ophthalmol Vis Sci*. 2017;58:6221–6231.
23. Aspinall PA, Johnson ZK, Azuara-Blanco A, Montarzino A, Brice R, Vickers A. Evaluation of quality of life and priorities of patients with glaucoma. *Invest Ophthalmol Vis Sci*. 2008; 49:1907–1915.
24. Nelson P, Aspinall P, O'Brien C. Patients' perception of visual impairment in glaucoma: a pilot study. *Br J Ophthalmol*. 1999;83:546–552.
25. Ramulu P. Glaucoma and disability: which tasks are affected, and at what stage of disease? *Curr Opin Ophthalmol*. 2009; 20:92–98.
26. Hu CX, Zangalli C, Hsieh M et al. What do patients with glaucoma see? Visual symptoms reported by patients with glaucoma. *Am J Med Sci*. 2014;348:403–409.
27. Zhang X, Loewen N, Tan O, et al. Predicting development of glaucomatous visual field conversion using baseline Fourier-domain optical coherence tomography. *Am J Ophthalmol*. 2016;163:29–37.
28. Distante P, Lombardo S, Verticchio Vercellin AC, et al. Structure/function relationship and retinal ganglion cells counts to discriminate glaucomatous damages. *BMC Ophthalmol*. 2015;15:185.
29. González-García AO, Vizzeri G, Bowd C, Medeiros FA, Zangwill LM, Weinreb RN. Reproducibility of RTVue retinal nerve fiber layer thickness and optic disc measurements and agreement with stratus optical coherence tomography measurements. *Am J Ophthalmol*.147:1067–1074.e1.
30. Demirkaya N, van Dijk HW, van Schuppen SM, et al. Effect of age on individual retinal layer thickness in normal eyes as measured with spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013;54:4934–4940.
31. Bartholomew AJ, Lad EM, Cao D, Bach M, Cirulli ET. Individual differences in scotopic visual acuity and contrast sensitivity: genetic and non-genetic influences. *PloS One*. 2016;11: e0148192.
32. Ooto S, Hangai M, Tomidokoro A, et al. Effects of age, sex, and axial length on the three-dimensional profile of normal macular layer structures. *Invest Ophthalmol Vis Sci*. 2011; 52:8769–8779.
33. Arvind H, Klistorner A, Grigg J, Graham SL. Low-luminance contrast stimulation is optimal for early detection of glaucoma using multifocal visual evoked potentials. *Invest Ophthalmol Vis Sci*. 2011;52:3744–3750.
34. Ashimatey BS, King BJ, Swanson WH. Retinal putative glial alterations: implication for glaucoma care. *Ophthalmic Physiol Opt*. 2018;38:56–65.
35. Nützi C, Schötzau A, Grieshaber MC. Structure and function relationship of activated retinal glia in primary open-angle glaucoma patients. *J Ophthalmol*. 2017;2017:7043752.
36. Vecino E, Rodriguez FD, Ruzafa N, Pereiro X, Sharma SC. Glia-neuron interactions in the mammalian retina. *Prog Retin Eye Res*. 2016;51:1–40.
37. Sofroniew MV. Molecular dissection of reactive astrogliosis and glial scar formation. *Trends Neurosci*. 2009;32:638–647.
38. Contín MA, Benedetto MM, Quinteros-Quintana ML, Guido ME. Light pollution: the possible consequences of excessive illumination on retina. *Eye (Lond)*. 2016;30:255–263.
39. Denniss J, Turpin A, McKendrick AM. Visual contrast detection cannot be predicted from surrogate measures of retinal ganglion cell number and sampling density in healthy young adults. *Invest Ophthalmol Vis Sci*. 2014;55:7804–7813.

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