Experimental Eye Research xxx (2014) 1–6

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Age-related macular degeneration and cognitive impairment show similarities in changes of neutral lipids in peripheral blood mononuclear cells

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ARTICLE INFO

Article history:
Received 22 November 2013
Accepted in revised form 21 April 2014
Available online xxx

Keywords:
age-related macular degeneration
cholesterol-ester
cognitive impairment

ABSTRACT

Starting from previous studies showing that patients with cognitive deficit present neutral lipids (NLs) accumulation in cytoplasm of their peripheral blood mononuclear cells (PBMCs) and considering that there is epidemiological evidence linking age-related macular degeneration (AMD) to cognitive deficit, the first purpose of this study was to test whether neutral lipids also accumulated in PBMCs from AMD subjects. Moreover, the impact of statin use on AMD was explored and whether such use in AMD subjects was associated with NLs accumulation in PBMCs. The study was conducted on 222 subjects: 136 AMD (36 of which – 26.5% – using statins], 48 cognitive deficit (20 of which – 41.7% – using statins) and 38 healthy controls (4 of which –10.1% – using statins), AMD lesions were assessed from color fundus photographs. Mini-mental state examination (MMSE), demographics, lifestyle factors and medical history were collected at interview. MMSE score was categorized as normal (24–30), and impaired (<24), NLs content was evaluated by oil red 0 (ORO) staining method. ORO determination showed that neutral lipids were generally absent or very low (score between 0 and 1) in healthy controls while most of PBMCs from cognitive deficit and AMD had ORO staining levels scoring 2–4. Post hoc analysis (Bonferroni) in a one-way ANOVA revealed that ORO score was significantly higher in cognitive deficit and AMD subjects compared to healthy controls and in cognitive deficit compared to AMD. Bonferroni-test also showed that AMD subjects had significantly lower total cholesterol (TC) levels compared to healthy controls while high density lipoprotein-cholesterol (HDL-C) did not reach statistical significance. The results also revealed a significant higher number of statin-users in AMD compared to healthy controls. Likewise when cognitive deficit vs healthy controls was analyzed, the number of statin users were found to be significant higher in cognitive deficit than in healthy controls. There were no significant differences in statin use between AMD and cognitive deficit. Compared to healthy controls, statin use in cognitive deficit and AMD groups was significantly associated with ORO scores of 2–4. This data supports the hypothesis that AMD and cognitive deficit share similar complex pathophysiology and risk factors including NLs accumulation in their PBMCs, although this does not necessarily imply that one disease causes the other. In addition, they provide further evidence that statin use may increase the risk of AMD.

1. Introduction

Age-related macular degeneration (AMD) and Alzheimer's disease (AD) are chronic degenerative disorders characterized by extracellular amyloid deposits (Sarks, 1980; Green and Enger, 1993; Baker et al., 2009; Ding et al., 2009; Butterfield et al., 2002; Kaarniranta et al., 2011). The pathophysiology of both diseases is largely unknown, but the current opinion is that they are genetically complex disorders possibly caused by a variety of molecular

Uncommon abbreviations: CEs, cholesterol esters; NLs, neutral lipids; CON, healthy controls; CD, cognitive deficit; AMD1, early or intermediate stages of AMD; AMD2, advanced AMD; OR, Odds ratio; TC, total cholesterol.

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http://dx.doi.org/10.1016/j.exer.2014.04.017
0014-4835/

defects. In this respect, numerous recent studies provide evidence that these two age-related illnesses have a common cause and origin even if they're happening in two different organs and with very different effects (Butterfield et al., 2002; Kaarniranta et al., 2011; Dentchev et al., 2003; Johnson et al., 2002; Luibl et al., 2006; Malek et al., 2005). For example, a common characteristic of these two diseases is the injury and death of neural cells, which may result in an irreversible loss of neuron function (Adler et al., 1999; Niikura et al., 2006). The formation of insoluble extracellular deposits consisting of misfolded, aggregated protein, mainly amyloid beta (AP) is another common characteristic. In aging human eyes and in eyes affected by AMD, these deposits called drusen, are found beneath the basal membrane of the retinal pigmented epithelium (RPE) and the inner collagenous layer of the Bruch membrane. Although the exact role of drusen in the pathogenesis of AMD is still unclear, these deposits are considered the initial stage of the disease. It has been previously shown that the presence of large and soft drusen is related to elevated cholesterol deposits, mainly in the esterified form (Curcio et al., 2005a, 2005b) and that the lipid content of drusen and its diffuse accumulation in the Bruch's membrane of the retina may be responsible for the loss of vision (Esenwah, 2010). One of the consequences of accumulation of cholesterol esters (CEs) at the basal face of the RPE would be a decreased nutrient intake by the neural retina, resulting in compromised retinal function (Bretillon et al., 2008a). Starting from our previous studies showing that AD patients present neutral lipids (NLs) accumulation in cytoplasm of their peripheral blood mononuclear cells (PBMCs) (Pani et al., 2009a; Mandas et al., 2012), in the present study neutral lipids (NLs) content in PBMCs from healthy controls (CON), AMD and subjects with cognitive deficit (CD) has been compared. Our aim was to establish whether NL accumulation in PBMCs may be another common characteristic shared by AMD and CD. Deregulation of lipid metabolism and transport, either on a local and/or systemic level has been also reported to contribute to AMD, and statin use has been associated with a decreased rate of macular degeneration (Wilson et al., 2004; Kishan et al., 2011). Despite the numerous studies on statins and AMD, contradictory results have so far been reported. Some studies revealed, in fact, that statin use was associated with a decreased rate of AMD (Vingerling et al., 1995; Wilson et al., 2004; Kishan et al., 2011), others that statins had no effect (Gehlbach et al., 2012) and still others that statins increased the risk of AMD (VanderBeek et al., 2013). Therefore, in this study, the impact of statin use on AMD was also explored and whether such use in AMD subjects was associated with PBMC-NLs accumulation.

2. Materials and methods

2.1. Subjects

In total, 222 subjects were enrolled in this study: 136 patients with AMD (any form, any-AMD), 48 patients with CD and 38 CON. All subjects were recruited between February 2007 and October 2011 at the University Eye Clinic of Cagliari, Italy. CON group consisted of normal individuals attending the outpatient clinic because of refractive disorders, or post-cataract follow-up visits. To maximize the reliability of our study, we deliberately selected age-matched controls. Written informed consent was obtained from all participants. Detailed patient history was recorded with the use of a questionnaire focusing on known or suspected non-genetic risk factors of any-AMD such as cigarette smoking, exposure to blue light, and medical history of acute myocardial infarction, ischemic heart disease and deep venous thrombosis. Color fundus photographs of the macular area were taken from both eyes in all subjects. Fluorescein angiography was used to investigate patients with exudative AMD.

Any-AMD participants were classified into 2 subgroups based on the Age-Related Eye Disease Study Group (AREDS) classification (Ferris et al., 2005): 98 patients with early or intermediate stages of AMD (AMD1), and 38 patients with advanced AMD (AMD2). All enrolled subjects (patients and controls) underwent a Mini Mental State Examination (MMSE) corrected for age and education, to identify cognitive deterioration, 30 correct-answer points indicating cognitive deficit absence, and 0, maximum cognitive deficit. Subjects with a MMSE score <24 were considered cognitively impaired (Folstein et al., 1975). No macular abnormality in either eye was observed in CD and CON groups, while among AMD 1, two patients had MMSE score <24. Patients or CON with other diseases interfering with reliable evaluation of AMD were not included in the study. Subjects on statin medication were defined as those using 3-hydroxy-3-methyl-glutaryl-CoA reductase inhibitors including more lipophilic statins such as simvastatin and less lipophilic, atorvastatin, rosuvastatin and pravastatin for at least three years.

2.2. Isolation of PBMCs

For the isolation of PBMCs, 500 μL of Ficoll were placed into 1.5 mL eppendorf tubes and an equal amount of anticoagulated whole blood was carefully layered on top of the Ficoll. The tubes were then centrifuged at 5000 × *g* for 10 min and the white ring in the middle layer containing the PBMCs removed and carefully plated in 6-well tissue culture plates without any additional growth factors.

2.3. Plasma lipid profile

Total cholesterol (TC) and high density lipoprotein-cholesterol (HDL-C) content was determined in plasma by using routine colorimetric enzymatic procedures (Sciavo Diagnostics International S.r.l. Sovicille, Italy).

2.4. NL content determinations

For NL measurements, PBMCs isolated as above were washed three times with PBS and fixed by soaking in 10% formalin. The cells were then treated with isopropyl alcohol (60%), washed again in buffer and stained with Mayer hematoxylin solution and Oil Red O (ORO), for the dictation of nuclei and intracellular NL droplets, respectively. ORO is a lipid-soluble dye which stains NLs, including cholesterol esters (CEs) and triglycerides, which appear as bright red spots in the cytoplasm, but not unesterified (free) cholesterol (Kruth and Fry, 1984). After staining, cells were imaged using an inverted phase microscope fitted with a digital camera. At least two different fields per sample were imaged and analyzed. The red intensity was scored on a semi-quantitative scale (from 0 to 4) by two masked observers: 0–1, indicated no staining or rare positive cells; 2, focal staining or faint diffuse staining clearly visible at low power staining; 3, multifocal staining or moderate diffuse staining; and 4, intense diffuse staining.

2.5. Statistical analysis

Quantitative variables were shown as mean ± standard deviation (M ± SD). Comparisons among these variables were made by Student test or by an analysis of variance (ANOVA). If a main effect was observed by ANOVA, a post-hoc test (Bonferroni) was applied to identify significant differences among categories. Odds ratios (ORs), confidence intervals and chi-square (χ^2) tests were

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performed to identify statistically significant associations. A statistical significance level of $P < 0.05$ was used for all tests. All statistical tests were made using Excel's data analysis tool.

3. Results

This study includes a total of 222 participants of which 136 were affected by any-AMD (98 AMD1 and 38 AMD2), 48 by CD and 38 were CON. The baseline characteristics of the study participants are shown in Table 1. No significant group differences in age were found ($P > 0.05$) (Table 1). Both CON and AMD experimental groups had significantly higher mean scores of MMSE compared to CD group ($P < 0.05$). Interestingly, only two subjects, both with AMD1, of the any-AMD group have been found to have a MMSE score <24. Using ANOVA it was also found that TC significantly differ among the groups (P<. Post hoc analysis (Bonferroni)) in a one-way ANOVA showed that AMD (AMD 1 and AMD2) subjects had significantly lower TC levels compared to CON. HDL-C did not reach statistical significance for any of the estimated groups (Table 1). We previously reported that NLs consisting mainly of CEs are increased in peripheral cells from CD (mainly AD) subjects compared to CON (Pani et al., 2009a, 2009b; Mandas et al., 2012). In those studies, we utilized different methodological approaches to assess NL content, including solvent extraction, thin-layer chromatography (TLC), [^{14}C] acetate labeling, Nile Red and oil red O (ORO) staining; all of them revealing high levels of NLs in PBMCs from AD patients. Among these techniques ORO staining was the cheapest, fastest, and easiest to perform, and was one that has required less material. Therefore, we proposed the determination of NLs in PBMCs by ORO staining, as a useful tool for early AD detection in clinical practice (Pani et al., 2009a, 2009b; Mandas et al., 2012). Accordingly to that, in a recent study (Mehlem et al., 2013) details for an easy and optimal estimation of tissue lipid content and distribution by using ORO staining were also presented. In order to examine whether cytoplasmic NL accumulations were observable also in AMD subjects, freshly isolated PBMCs from CD, AMD and CON subjects were stained with ORO. Using this method, it was found that NLs in CON were generally absent or very low (score between 0 and 1), while PBMCs from CD and AMD had general ORO staining levels scoring 2–4 (Fig. 1A). When NL levels in ORO stained PBMCs from CON, CD and AMD (AMD1 and AMD2) were semi-quantified on the basis of the intensity of the lipid bound red color, statistical analysis performed by using the one-way ANOVA test showed highly significant differences between the groups ($P < 0.05$). Post hoc analysis (Bonferroni) in a one-way ANOVA revealed that ORO score was significantly higher in CD and AMD (AMD1 and AMD2) subjects compared to CON; in CD compared to AMD (AMD1 and AMD2) as well as in AMD2 compared to AMD1 (Fig. 1B) ($P < 0.05$). It is interesting to note that, freshly isolated (un-stimulated) PBMCs from AMD and CD tend to aggregate in vitro in small-to-medium clusters (Fig. lA) hence resembling cultured PBMCs after mitogen activation with phytohemagglutinin (Pani et al., 2009a; Mandas et al., 2012). These aggregates formation are not detected in cultures of un-stimulated CON cells (Fig. 1A). Among AMD subjects, 72% (98 subjects) had early or intermediate stages of AMD (AMDl) and 28% (38 subjects) had advanced AMD (AMD2) (Table 2). Seventy percent of subjects with AMDl and 84% of that with AMD2 had ORO scores ranging from 2 to 4 compared to 18% of CON (Table 2). There were also significant differences in the number of subjects with ORO scores of 2–4 between CD (higher) and Any-AMD (lower) ($P < 0.05$). No significant difference between the number of AMDl vs AMD2 and CD vs AMD2 in terms of ORO score was observed. The calculated values of χ^2 and OR between the various groups are reported in Table 2. Given the compelling evidence that atherosclerosis and AMD share a similar pathogenic process, as a final point, an existing association between statin use and AMD has been detected. Here, it was not possible to provide data regarding specific type of statins or dosage used; however, in a recent study, any statistically significant difference in MMSE scores among subjects treated with different types of statins was recorded (Mandas et al., 2014). The number and the percentage of both statin and non-statin users among CON, CD and AMD groups and the calculated values of χ^2 and OR are reported in Table 3. The results revealed a significant higher number of statin-users in Any-AMD group compared to CON. Likewise when CD vs CON was analyzed, the number of statin users was found to be significant higher in CD than in CON. There were no significant differences in the number of statin users between any-AMD and CD and AMDl and AMD2 groups. Table 3 also shows that no significant difference between statin users and non-users for TC and HDL-C levels occurs in all study groups. The number of statin and non-statin users divided in two ORO score ranges (0–1 and 2–4) and the calculated values of χ^2 and OR are reported in Table 4. The results showed that statin use in CD and Any-AMD groups was significantly associated with ORO scores of 2–4. No significant difference with regard to ORO scores was observed between statin users of any-AMD and CD groups and of those AMD1 and AMD2. Calculation of OR was not possible for CD or AMD2 subjects taking statins with ORO scores of 0–1 ($n = 0$).

4. Discussion

It had been previously reported that freshly isolated PBMCs from AD patients and some of their first degree relatives are characterized by alterations in cholesterol esterification leading to an accumulation of NLs (mainly CEs) in their cytoplasm (Pani et al., 2009b; Mandas et al., 2012). Here, using a semi-quantitative analysis based on ORO staining to determine NL content in naive PBMCs from AMD, CD and CON, a greater intensity of ORO staining (score 2–4) was associated not only with CD but also with AMD. As such, these data point to a role for NLs in the pathogenesis of AMD and CD suggesting that although AMD and CD are not directly related, in the sense that one person must necessarily suffers of both diseases, accumulation of NLs in PBMCs may be a common characteristic shared by the two diseases. These results also support our previous conclusions that abnormalities of cholesterol esterification may represent a phenotype predisposed to the development of a

Table 1
Characteristics of study participants. The values are expressed as M ± SD. Differences that are statistically significant by ANOVA are indicated in bold. TC (total cholesterol).

Participants $n = 222$	1	2	3	4	5	ANOVA P-value	Bonferroni At $P < 0.05$
	CON $n = 38$	CD $n = 48$	Any-AMD $n = 136$	AMD1 $n = 98$	AMD2 $n = 38$		
Age (y)	75.3 ± 5.5	75.3 ± 5.3	76.5 ± 9.7	77.5 ± 8.8	74.1 ± 11.4	>0.05	>0.05
Sex (F/M)	21/17	35/13	61/75	44/54	20/18		
MMSE (score)	27.7 ± 1.6	20.4 ± 2.7	27.8 ± 1.5	27.6 ± 1.3	27.9 ± 1.6	**<0.0005**	2 vs 1,3,4,5
TC (mg/dl)	186.0 ± 39.4	169.4 ± 45.3	153.0 ± 43.1	153.1 ± 43.65	152.8 ± 43.05	**<0.0005**	1 vs 3,4,5
HDL-C (mg/dl)	50.7 ± 8.2	48.3 ± 21.6	52.6 ± 15.5	53.4 ± 14.7	51.4 ± 16.6	>0.05	>0.05

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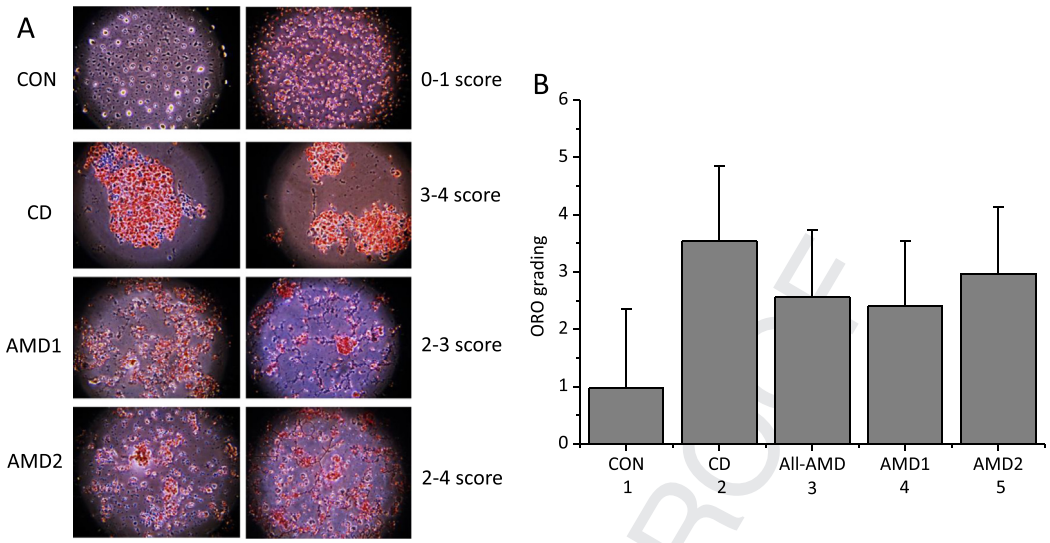

Fig. 1. ORO staining in CON, CD and AMD. A) Microphotographs of representative cells assigned to scores 0–4. B) Semiquantitative scoring of NLs with the use of ORO (M ± SD). Scores represent the proportion of cell cytoplasm occupied by lipid droplets Statistical analysis performed by using the one-way ANOVA test showed highly significant differences between the groups ($P < 0.0005$). Multiple comparisons were corrected by post hoc Bonferroni test. 1 vs 2,3,4,5; 2 vs 3,4,5; 4 vs 5, all have $P < 0.05$. Original magnifications: ×400.

number of neurodegenerative disorders, and consequently that inhibition of cholesterol esterification may be a way to control the progression of these illnesses (Pani et al., 2009b; Mandas et al., 2012; Anchisi et al., 2012). Accordingly, studies by Kovacs group found that inhibition of cholesterol esterification markedly reduced amyloid pathology in AD model mice (Puglielli et al., 2003; Hutter-Paier et al., 2004; Puglielli et al., 2004; Huttunen and Kovacs, 2008) suggesting a central role of CEs in AD pathogenesis. Another interesting finding of the current study was that PBMCs, freshly isolated from AMD and CD, have, contrary to CON, an increased tendency to in vitro spontaneous aggregation, in this resembling PBMCs mitogenically activated by treatment with phytohemagglutinin (Pani et al., 2009b). It is suggested that the presence of PBMC aggregates in vitro might be a sign of their activation in vivo, and thus that spontaneous aggregation of PBMCs in vitro could be considered a potential peripheral biomarker of neurodegenerative disorders including AMD and CD. In a recent observational study designed to provide further insights into the effect of the use of statins on cognitive functions in older people, it was found that statin users had lower MMSE than non-users. In the same study, it was demonstrated that NL content (detected by ORO staining) in PBMCs was greater in CD (determined by MMSE) compared to CON and that statin-users had higher ORO score than non-users (Mandas et al., 2014). This study also reported, with respect to use of water soluble (hydrophylic) or lipid soluble (lipophilic) statins, no significant difference in MMSE scores was observed. In recent years several reports have suggested that statins exert protective effects in AMD (McCarty et al., 2001; Wilson et al., 2004), however, existing studies have had limited power in reliably detecting or excluding an effect and have produced conflicting results (Maguire et al., 2009; Peponis et al., 2010; Gehlbach et al., 2012). It was therefore decided to exploit whether statin treatment was associated with any-AMD and to determine NL content by ORO staining in PBMCs from any-AMD subjects taking statins. It was found that the number of statin users was significantly higher in Any-AMD and in CD groups compared to CON. There was no

Table 2
Number and percentage of ORO stained CON, CD and AMD samples divided into two groups: one with 0–1 ORO scores, the other with 2–4 scores. Significant differences are in bold.

	ORO score		Total number	χ^2 (*P*-value) OR (95% CI)
	0–1	2–4		
CON	31 (81.6%)	7 (18.4%)	38	CD vs CON
				50.3 (<0.0005)
				66.4 (15.9–277.0)
CD	3 (6.3%)	45 (93.7%)	48	CD vs Any-AMD
				8.2 (<0.005)
				5.20 (1.5–17.8)
Any-AMD	35 (25.7%)	101 (74.3%)	136	Any-AMD vs CON
				39.3 (<0.0005)
				12.8 (5.2–31.6)
AMD1	29 (29.6%)	69 (70.4%)	98	CD vs AMD1
				10.3 (<0.005)
				6.3 (1.8–21.9)
AMD2	6 (15.8%)	32 (84.2%)	38	CD vs AMD2
				2.1 (>0.05)
				2.8 (0.6–12.1)
				AMD1 vs AMD2
				2.7 (>0.05)
				0.45 (0.2–1.2)

Table 3
Number and percentage of statin users among CON, CD and AMD groups. The data relative to total cholesterol (TC) and HDL-C are expressed as M ± SD. Significant differences are in bold.

	Statin use		*t*-test *P*-value	χ^2 (*P*-value) OR (95% CI)
	No	Yes		
CON	34 (89%)	4 (10.1%)		Any-AMD vs CON
TC (mg/dl)	187.6 ± 38.6	172.0 ± 49.6	0.65567	**4.3 (<0.05)**
HDL-C (mg/dl)	51.1 ± 8.5	48.0 ± 4.6	0.85437	3.1 (1.0–9.2)
CD	28 (58.3%)	20 (41.7%)		CD vs CON
TC (mg/dl)	165.6 ± 50.6	174.8 ± 37.3	0.23588	**10.2 (<0.005)**
HDL-C (mg/dl)	51.0 ± 24.9	44.6 ± 15.9	0.85945	6.1 (1.9–19.8)
Any-AMD	100 (73.6%)	36 (26.5%)		Any-AMD vs CD
TC (mg/dl)	153.3 ± 44.6	152.0 ± 39.3	0.56506	3.9 (>0.05)
HDL-C (mg/dl)	53.2 ± 14.1	51.0 ± 19.3	0.73281	0.5 (0.2–1.0)
AMD1	75 (76.5%)	23 (23.5%)		
TC (mg/dl)	150.7 ± 44.2	162.3 ± 42.2	0.13068	
HDL-C (mg/dl)	52.4 ± 11.9	57.3 ± 23.1	0.16699	
AMD2	25 (65.8%)	13 (34.2%)		AMD2 vs AMD1
TC (mg/dl)	158.0 ± 46.1	141.8 ± 35.4	0.92251	1.6 (>0.05)
HDL-C (mg/dl)	54.6 ± 17.6	44.8 ± 12.9	0.05677	1.7 (0.7–3.8)

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Table 4
Number of statin and non-statin users divided in two ORO score ranges. Significant differences are in bold.

	ORO score				χ^2 (*P*-value) OR (95% CI)
	0–1		2–4		
	Statins		Statins		
	No	Yes	No	Yes	
CON	29	2	5	2	CD-Yes vs CON-Yes **12.0 (<0.0005)**
CD	3	0	25	20	
Any-AMD	29	1	71	35	Any-AMD-Yes vs CON-Yes **11.6 (<0.0005)** 35.0 (2.1–5.7)
					Any-AMD-Yes vs CD-Yes 0.57 (>0.05)
AMD1	23	1	52	22	AMD1-Yes vs AMD2-Yes 0.6 (>0.05)
AMD2	6	0	19	13	

significant difference in the number of statin users between any-AMD and CD and AMD1 and AMD2 groups. Statin use in CD and Any-AMD groups was significantly associated with ORO scores of 2–4 in their PBMCs. No significant difference with regard to ORO scores was observed between statin users of any-AMD and CD groups and of those AMD1 and AMD2. These results suggest that treatment with statins might favor the occurrence of AMD. In addition, it was also indicated that similarly to CD, any-AMD subjects who are taking statins have a greater propensity to accumulate NLs in their PBMCs compared to CON. Until now, mechanisms able to validate possible cognitive dysfunction and other neurological consequences caused by statins have not yet been identified, thus a tentative explanation for the obtained results could be proposed. It is important to recall that cholesterol is typically an abundant component of plasma membranes of neurons including those of retina, where it helps to maintain their integrity, and plays a role in facilitating cell signaling (Fliesler and Bretillon, 2010). The needs of neurons for cholesterol and other membrane lipids are manifold, not only to support synapse formation, but to form and maintain intracellular membrane compartments as well as the plasma membrane, the axon, and dendritic arbors and to generate a dynamic supply of synaptic vesicles for neurotransmitter release. Cholesterol also has a unique role in the retina involving the generation and turnover of photoreceptor outer segment membranes. Contrary to the brain and with the exception of the optic nerve, neurons from the neural retina are not myelinated. Cholesterol appears to be broadly distributed in all layers of the neural retina (Bretillon et al., 2008a), although the inner plexiform layer is found to be relatively enriched in cholesterol compared with the outer segment layer (Francis, 1955). In the brain, cholesterol derives almost entirely from in situ synthesis, but it cannot be degraded, not crossing the blood brain barrier. In the neural retina, similar to the brain, cholesterol exists almost exclusively in the unesterified (free) form (Bretillon et al., 2008b; Fliesler and Bretillon, 2010). It has also been demonstrated that the mammalian retina has the capacity to synthesize its own cholesterol de novo. A single intravitreal injection of lovastatin was able to inhibit de novo cholesterol biosynthesis in the retina as determined by [^{3}H]acetate incorporation (Fliesler et al., 1993). Other neutral lipids, such as triglycerides, are extremely minimal if not completely absent in the retina (Bretillon et al., 2008b; Fliesler and Bretillon, 2010). These findings imply that a fine regulation of neural cholesterol dynamics is essential for basic functions, plasticity and behavior of neuronal cells (Anchisi et al., 2012). Hence, the demands on neuritic cells for cholesterol to achieve and maintain normal structure and function are remarkable. Since the most widely used inhibitors of cholesterol synthesis, known collectively as statins, cross the brain blood barrier (Botti et al., 1991), their long-term consumption, by reducing de novo cholesterol synthesis, may cause perturbations in cholesterol homeostasis resulting in progressive degeneration that significantly compromises the structure and function of neuronal cell types including those of the retina. In summary, our findings regarding the impact of statin use on AMD and CD strongly support the hypothesis that altering cholesterol metabolism can have profoundly deleterious effects in the neural tissue. Hence, maintaining normal steady-state levels of cholesterol in the brain and retina appears to be critical.

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Please cite this article in press as: Peiretti, E., et al., Age-related macular degeneration and cognitive impairment show similarities in changes of neutral lipids in peripheral blood mononuclear cells, Experimental Eye Research (2014), http://dx.doi.org/10.1016/j.exer.2014.04.017