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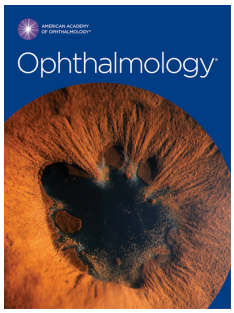

Increased High Density Lipoprotein-levels associated with Age-related Macular degeneration. Evidence from the EYE-RISK and E3 Consortia

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Running head: High HDL associated with AMD

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ABBREVIATIONS

AMD = Age-related macular degeneration; **ABCA1** = ATP-binding cassette (ABC) transporter A1; **(Apo)E =** Apolipoprotein E; **CETP** = Cholesteryl transfer protein; **LIPC** = Lipase C; **E3** = European Eye Epidemiology; **RPE** = retinal pigment epithelium; **VLDL** = very low density lipoproteins; **IDL** = intermediate density lipoproteins; **LDL** = low density lipoproteins; **HDL**= high density lipoproteins; **NMR**= nuclear magnetic resonance; **EPIC** = European Prospective Investigation into Cancer and Nutrition**; ALIENOR** = Antioxydants, Lipids Essentiels, Nutrition et maladies OculaiRes Study; **POLA**= Pathologies Oculaires Liées à l'Age Study**, EUGENDA** = European genetic database; **RS** = Rotterdam Study; **BMI** = body mass index; **PAMDI** = Prevalence of Age-related Macular Degeneration in Italy; **EDTA** = Ethylenediaminetetraacetic acid; **SNP**= single nucleotide polymorphism; **OR** = Odds Ratio; **HR** = Hazard Ratio; **LCAT** = lecithin-cholesterol acyltransferase;

This article contains additional online-only material. The following should appear online-only: Figure 4 , Tables 1, 4-19 and cohort descriptions.

ABSTRACT

Purpose: Genetic and epidemiologic studies have shown that lipid genes and High Density Lipoproteins (HDL) are implicated in age-related macular degeneration (AMD). We studied circulating lipid levels in relation to AMD in a large European dataset, and investigated whether this relationship is driven by certain sub fractions.

Design: (Pooled) analysis of cross-sectional data.

Participants: 30,953 individuals aged 50+ participating in the E3 consortium; and 1530 individuals from the Rotterdam Study with lipid sub fraction data.

Methods: In E3, AMD features were graded per eye on fundus photographs using the Rotterdam Classification. Routine blood lipid measurements were available from each participant. Data on genetics, medication and confounders such as body mass index, were obtained from a common database. In a subgroup of the Rotterdam Study, lipid sub fractions were identified by the Nightingale biomarker platform. Random-intercepts mixed-effects models incorporating confounders and study site as a random-effect were used to estimate the associations.

Main Outcome Measures: early, late or any AMD, phenotypic features of early AMD, lipid measurements.

Results: HDL was associated with an increased risk of AMD, corrected for potential confounders (Odds Ratio (OR) 1.21 per 1mmol/L increase (95% confidence interval[CI] 1.14-1.29); while triglycerides were associated with a decreased risk (OR 0.94 per 1mmol/L increase [95%CI 0.91-0.97]). Both were associated with drusen size, higher HDL raises the odds of larger drusen while higher triglycerides decreases the odds. LDL-cholesterol only reached statistical significance in the association with early AMD (p=0.045). Regarding lipid sub fractions: the concentration of extra-large HDL particles showed the most prominent association with AMD (OR 1.24 [95%CI 1.10-1.40]). The *CETP* risk variant (rs17231506) for AMD was in line with increased-HDL levels ($p=7.7x10^{-7}$); but *LIPC* risk variants (rs2043085, rs2070895) were associated in an opposite way ($p=1.0x10^{-6}$ and $1.6x10^{-4}$).

Conclusions: Our study suggests that HDL-cholesterol is associated with increased risk of AMD and triglycerides negatively associated. Both show the strongest association with early AMD and drusen. Extra-large HDL sub fractions seem to be drivers in the relation with AMD, variants in lipid genes play a more ambiguous role in this association. Whether systemic lipids directly influence AMD or represent lipid metabolism in the retina remains a question to be answered.

Word count: 350

Keywords: Age-related macular degeneration , lipids, high-density lipoproteins, cholesterol, E3 Consortium

INTRODUCTION

Age-related macular degeneration (AMD) is a leading cause of blindness in the developed world with 10.4 million sufferers worldwide in 2015[1]. It is a multifactorial disease affecting the elderly involving genetics and lifestyle factors. The diagnosis of AMD is based on imaging of the retina with drusen as the hallmark of early disease, and chorioretinal neovascularization and atrophy of the retinal pigment epithelium (RPE) are indicative of late disease. The number of drusen and total drusen area are prominent predictors of progression of the early stages of AMD[2, 3].

Drusen are lipid-rich, protein-containing deposits that accumulate between the RPE and Bruch's membrane. The accumulation of drusen shows resemblance to the formation of atherosclerotic plaques[4] seen in cardiovascular disease, with a similar composition of proteins and protein complexes such as apolipoprotein (Apo)E, cholesterol esters and complement proteins.[5, 6] The lipid load in drusen is as high as 40%[7], and is thought to be partly derived from the systemic circulation. This triggered many studies to evaluate the relationship between serum or plasma lipids and AMD.[8-12] Some found associations with various serum or plasma lipid levels and drusen or AMD[11-18], but results were mainly weak and inconsistent. As a biological explanation is lacking, the relationship remains unsettled but intriguing.

Genetically, lipid metabolism is also involved in AMD. Genetic associations have been established for four genes encoding components of the HDL metabolism: *ABCA1*, *CETP*, *APOE* and *LIPC*[19-25]. The ATP-binding cassette (ABC) transporter A1 (ABCA1) is a cellular cholesterol efflux pump leading to formation of nascent HDL. ApoE, encoded by the *APOE* gene, facilitates cholesterol uptake by HDL. Cholesteryl transfer protein (CETP) exchanges cholesteryl esters and triglycerides between HDL and other lipoproteins, and thereby influences HDL particle size.[26] Lastly, hepatic lipase encoded by the *LIPC* gene hydrolyzes triglycerides and phospholipids in lipoproteins[27] and thereby partly converts very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL) to low density lipoproteins (LDL)[20] and plays a role in altering the HDL contents.

The European Eye Epidemiology (E3) consortium within the European EYE-RISK project enabled us to investigate the relationships between systemic lipids levels, lipid genes, and AMD using a very large data set. With nuclear magnetic resonance (NMR) spectroscopy we studied these relationships in greater detail to investigate which particles are driving potential associations.

METHODS

Study population:

Routine blood lipid measurements

Fourteen studies from France, Germany, Italy, the Netherlands, Norway, Portugal and United Kingdom participating in the E3 consortium enrolled in the current study (cohort descriptions available at External link http://www.aaojournal.org). E3 consists of European studies with epidemiologic data on common eye disorders; a detailed description on the studies included in the consortium has been published elsewhere.[28] All studies with gradable macular fundus photographs (*N*=30,953 participants) aged 50 years and over contributed their data to the EYE-RISK database version 4.0. Studies were population-based cohort studies except for CRETEIL and EUGENDA which are clinic-based studies. Routine blood lipid measurements and AMD outcomes of the same visit were used for this analysis; for TwinsUK the closest visit to capturing of the retinal fundus photos was used. All studies were performed in accordance with the Declaration of Helsinki for research involving human subjects and the good epidemiological practice guideline.

Detailed lipid analyses

The population-based Rotterdam Study (RS) I provided data on lipid sub fractions which were determined at visit 4. Descriptive statistics of this cohort are shown in **Supplementary Table 1** (available at External link http://www.aaojournal.org).

Clinical examination:

AMD features were graded per eye on fundus photographs by experienced graders or clinicians; the most severe AMD grade classified the AMD status of the person. When needed, photographs were regraded by expert graders from Moorfields Eye Hospital and the Rotterdam Study to harmonize the outcome. AMD status was determined for all included studies using the Rotterdam Classification as previously described.[29] In brief, grade 0 or 1 are considered no AMD; grade 2 and 3 with soft indistinct drusen, reticular drusen or distinct drusen with pigmentary changes as early AMD, and grade 4 with Geographic Atrophy or Choroidal Neovascularization as late AMD. The area of the Early Treatment Diabetic Retinopathy Study grid covered by drusen was estimated in RS I visit 4 per grid circle, and calculated using previously defined harmonization criteria[30]. Medication use and lifestyle factors

including smoking habits were assessed by questionnaire; lipid measurements and other clinical determinants such as hypertension, body mass index (BMI), diabetes mellitus were examined at each individual research center (**Supplement cohort descriptions** available at External link http://www.aaojournal.org). Fasting blood draws were taken in all studies except for EUGENDA, MARS, and the Tromsø Eye Study, which drew blood samples non-fasting. Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured in plasma (POLA, PAMDI, Montrachet-3C, CRETEIL) or in serum (remaining studies) using standard operating procedures. When LDL was not measured and triglycerides were below 4.52mmol/L, a proxy was calculated using the Friedewald formula[31]: LDL-cholesterol = Total cholesterol – HDL cholesterol – (Total triglyceride ÷ 2.19); only positive values entered the analysis.

Nuclear magnetic resonance (NMR) metabolomics analysis

Lipid sub fractions were measured with the Nightingale's NMR-based biomarker platform in fasting Ethylenediaminetetraacetic acid (EDTA) plasma samples (Nightingale Ltd, Helsinki, Finland). These measurements cover multiple metabolic pathways, including lipoprotein lipids and subclasses, fatty acids, amino acids, and glycolysis-related metabolites. The NMR-based metabolic profiling has previously been described in detail[32] and has been used in multiple large-scale epidemiological and genetic studies[33-36].

Genetic analyses

The Alienor-3C study and Montrachet-3C study were genotyped with the Illumina Human 610-Quad BeadChip and imputed with the 1000 Genomes Phase I integrated variant set (March2012) using Shapeit v2.r727 (https://mathgen.stats.ox.ac.uk/genetics_software/shapeit/shapeit.html) for pre-phasing and Impute2 v2.3 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html) for imputation. Rotterdam Study I, II and III were genotyped using the Illumina 550K, 550k due/610K Illumina arrays. The genotypes were imputed with the 1000 Genomes (Phase 1 version 3) reference panel using the Markov chain Haplotyping (MaCH)/minimac software[37-39]. The EUGENDA study was genotyped with a custom-designed Illumina HumanCoreExome array within the International AMD Genetics Consortium (IAMDGC). Details regarding the design of this array, annotation, imputation and quality control of the genotypic data have

been previously described[19]. All cohorts applied similar quality control procedures to genotype data prior to analysis, imputation quality was r^2>0.3.

A total AMD genetic risk score was calculated using 33 out of the 52 known AMD risk variants[19] available in the EYE-RISK database version 4.0, see also subscript **Supplementary Table 19** (available at External link http://www.aaojournal.org). Genetic allele dosage was annotated as 0 for non-carriers, 1 for heterozygotes, and 2 for homozygotes. The genetic risk score was composed by calculating the sum of the betas of independent risk variants. The score was standardized and added as a covariate in a linear regression analysis with AMD as the dependent variable. The linear regression was corrected for age, sex, lipid lowering drugs and study site. The effect of individual lipid-related single nucleotide polymorphisms (SNP) on each lipid level or lipid sub fraction was assessed in a mixed-effects regression correcting for age, sex, lipid lowering drug usage, plasma or serum, fasting state and using study site as a random effect term. The p-value threshold for these analyses were 0.05/60 = 0.00083 ($8.3x10^{-4}$) after Bonferroni correction.

Statistical analysis

The outcome variable was presence of early or late AMD versus no AMD. Differences in baseline characteristics were evaluated with a Wald test using a logistic regression analysis, adjusting for age, gender, and study site. Analyses were conducted on complete data. Odds ratios (OR) for the routine blood lipid measurements were calculated using random-intercepts mixed-effects logistic regression models, including study site as a random effect term to allow for variability between study sites. The study site specific fixed effects estimates were transformed to their marginal counterparts as described by Heagerty and Zeger[40].

Association of HDL-cholesterol with AMD characteristics (presence of various drusen sizes, hyper- or hypopigmentation) were calculated in a univariate logistic regression analysis for the worse eye, defined as the eye with the most severe lesions of each AMD characteristic, correcting for age, sex, lipid lowering drugs usage and study site. The linear regression for HDL-cholesterol and drusen area was calculated in the Rotterdam Study I visit 4 only.

For the analysis on lipid sub fractions, all sub fractions were +1 log transformed and scaled to make comparable measurements. Association magnitudes were reported in units of standard deviation (SD) or odds ratio (OR) change per 1-SD increase in each metabolite, as previously suggested by others[34, 35]. To account for the correlation between lipid sub fractions, the eigenvalues were calculated as proposed by

Li and Ji[41] on the SNPSpD online interface[42]. Bonferroni correction was applied to correct for multiple testing using the eigenvalues to calculate the p-value threshold (p-value=0.001087). To test for differences between AMD stage and the mean of the lipid sub fractions, a Welch test was performed on the total of all age categories. The Welch test was chosen since homogeneity of variance was violated between the AMD severity classes. The post hoc Games-Howell test was used to investigate differences between the mean of the No AMD and Late AMD groups.

Mixed-effects logistic regression models were performed with R package lme4[43] and mixed-effects regression models with nmle[44] (R Core Team (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/); Welch-test and genetic risk scores with SPSS (IBM Corp. Released 2012 IBM SPSS Statistics for Windows, Version 24.0 Amonk, NY: IBM Corp). Graphical outputs were constructed with GraphPad Prism 7 (GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com").

RESULTS

We identified a total of 4,730 individuals with early AMD; 2,441 with late AMD; and 23,782 non-affected persons. The baseline characteristics of these participants are summarized in **Table 2**. AMD cases and controls differed in age, sex, BMI, lipid lowering drug use, and smoking, in accordance with the known AMD risk profile.

Routine blood lipid measurements and AMD in the E3 consortium

Next, we examined lipid levels in the entire study population. Mean levels of all lipids were within physiologic limits: mean total cholesterol ranged between studies from 5.1 mmol/L to 5.8 mmol/L; mean HDL-cholesterol from 1.4 mmol/L to 1.9 mmol/L. Mean LDL-cholesterol ranged from 3.0 mmol/L to 3.8 mmol/L; and mean triglycerides from 1.2 mmol/L to 1.7mmol/L. A fifth of the study population had collected only non-fasting blood samples; in these, the mean levels were similar but on the higher range of total cholesterol and triglycerides; 5.7 mmol/L for total cholesterol and 1.7mmol/L for triglycerides. Mean HDL-cholesterol was on the lower range with 1.5mmol/L and LDL-cholesterol was very comparable with 3.5 mmol/L.

Table 3 shows the association between lipid levels and AMD adjusted for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site in the E3 consortium. Analyses for late AMD were also corrected for diabetes. Total cholesterol was not associated with any of the AMD outcomes. Higher HDL-cholesterol was associated with an increased risk of any AMD, and risk estimates were slightly higher for early AMD (OR 1.34 per 1 mmol/L increase), than for late AMD (OR 1.12 per 1 mmol/L increase), but had overlapping confidence intervals. LDL-cholesterol and triglycerides were inversely associated with early (OR 0.96 and OR 0.88 per 1 mmol/L increase, respectively) and any AMD (OR 0.98 and OR 0.94 per 1 mmol/L increase, respectively), but effect sizes were smaller than for HDL-cholesterol. Sensitivity analysis on fasting and non-fasting sampling methods and sex showed no interaction or it showed interaction but with similar point estimates of the odds ratios which made sampling effect or confounding unlikely. However, a sensitivity analysis on plasma or serum sampling methods did show a change in direction of effect of triglycerides measured in plasma, although not statistically significant (**Supplementary Table 4-10**, available at External link http://www.aaojournal.org). To investigate whether observed associations were the result of preferential survival of elderly without cardiovascular disease, we repeated the analyses in various age strata (**Supplementary Table 11-13**, available at External link http://www.aaojournal.org). Even in those aged 65 years or younger with AMD, HDL-cholesterol was significantly associated with increased risk of AMD (OR 1.19 per 1 mmol/L increase, p-value 0.02). LDL-cholesterol was inversely associated with AMD (OR 0.93 per 1 mmol/L, p-value 0.02), associations with triglycerides became insignificant.

Routine blood lipids measurements and early AMD phenotype

As the association between HDL-cholesterol and AMD was most pronounced in those with early AMD, we performed more detailed analyses using the various early AMD features as outcomes. Effects of HDL-cholesterol and triglycerides became larger with increasing drusen size, **Figure 1**. Likewise, higher HDL levels were associated with greater drusen area (beta 0.014; P-value=0.001); higher triglyceride levels were associated with lower drusen area. Correcting for smoking did not change these results (data not shown). Lipids were not statistically significantly associated with pigmentary changes, and total cholesterol and LDL-cholesterol were not associated with any early AMD characteristic (**Supplementary table 15 and 16**, available at External link http://www.aaojournal.org).

Lipid sub fractions in the Rotterdam Study

To explore whether the association between HDL-cholesterol, triglycerides, and AMD was driven by specific lipid sub fractions, we examined lipid sub fractions with NMR in the Rotterdam Study-I **Figure 2** (**Supplementary table 14**, available at External link http://www.aaojournal.org). The concentration of extra-large HDL particles (XL-HDL-P) was most significantly associated with any AMD, particularly the sub fractions of phospholipids and total lipids within extra-large HDL particles. These sub fractions are highly correlated (Pearson correlation >0.97). Next, total cholesterol and free cholesterol in the small VLDL were significantly associated, as well as the ratio ApoB:ApoA1, with a Pearson correlation ranging between 0.93 and 0.87. No other metabolites were significantly associated with AMD. Correcting for smoking did not change these significant results (data not shown). The ApoB-ApoA1 ratio is a surrogate for the LDL/HDL ratio with a small ratio suggesting a high level of HDL compared to LDL lipoproteins.
These associations show a dose dependent relation with AMD stages from the Rotterdam Classification **Figure 3**. To test if the mean of the lipid sub fractions per AMD stage differed statistically, we performed a Welch test, which was significant for each of the six sub fractions. The sub fractions related to HDL also showed a statistically significant difference in the Games-Howell post hoc test comparing the mean of those with no AMD with late AMD; p-value=0.01 for the concentration of extra-large HDL, p-value=0.02 for phospholipids in extra-large HDL and the p-value=0.01 for total lipids in extra-large HDL. The Games-Howell post-hoc test was not significant in the other three sub fractions, likely due to the small group size and variance in late AMD. (**Supplementary Figure 4** shows dose dependency per age category available at External link http://www.aaojournal.org.)

We also performed the analyses stratified for lipid-lowering drug use, which was reported by less cases (17.0%) than controls (24.2%) in the Rotterdam Study (p=0.02). Significance was found only in those not taking lipid-lowering drugs, and point estimates were highly similar to the overall group. (**Supplementary Table 17- 18**, available at External link http://www.aaojournal.org).

Lipid genes, lipid sub fractions and AMD

As genetic variants are an important cause of AMD, we investigated the relation between genes, lipids, and AMD. First, we investigated whether a genetic risk score with 33 SNPs covering all major AMD genes influenced lipid levels in the E3 consortium, and found with increasing genetic risk also an increase of HDL-cholesterol (p=0.03) (**Supplementary Table 19** available at External link http://www.aaojournal.org). Subsequently, we focused on the individual AMD lipid genes. In E3, the

CETP variant rs17231506 was positively associated with HDL-cholesterol levels and negatively associated with LDL-cholesterol, while both LIPC variants rs2043085 and rs2070895 were inversely associated with HDL-cholesterol. In addition, the APOE variant rs429358 was associated with decreased levels of total cholesterol, triglycerides, and LDL-cholesterol, but with increased levels of HDL-cholesterol. APOE variant rs73036519 had no significant effect on the routine lipid measurements or on the lipid sub fractions. ABCA1 variant rs2740488 only influenced total cholesterol (**Table 20A**). When restricting the analysis to lipid sub fractions in the Rotterdam Study (**Table 20B**), we found similar results for the CETP variant and the LIPC variants with all extra-large HDL sub fractions.

DISCUSSION

Routine blood lipid measurements

Based on pooled data of 30,953 participants from Western Europe, we have shown that high circulating HDL-cholesterol levels and low triglyceride levels are significantly associated with AMD. The magnitude of the effect was higher for early than for late AMD, and associations were related to drusen size and area. By focusing on lipid sub fractions, we revealed that extra-large HDL particles, small VLDL particles, and the ApoB-ApoA1 ratio, a surrogate for the LDL/HDL ratio, were dose-dependent drivers of this association. AMD risk variants in lipid genes did not provide a clear explanation, as in particular the variants in *LIPC* which increase the risk of AMD, decreased HDL-cholesterol in the systemic circulation.

Our results should be interpreted in light of the strengths and limitations of the study. The combined efforts of two European consortia enabled us to create a very large database providing the statistical power to resolve conflicting findings from previous studies. The detailed NMR lipid analysis in a subset created the opportunity to find the metabolic profile behind the lipid associations. A weakness of the consortium was the use of different protocols for blood sampling, definition of confounders, and AMD phenotyping. We addressed this issue by performing a stratified analysis on sampling methods, and found that only the associations for triglycerides changed direction of effect for plasma, albeit non-significantly. We harmonized all confounders as well as the criteria for early and late AMD, and corrected for study site in the mixed-effect models.

Many previous studies did not find a statistically significant association between lipids and AMD, but studies with the larger sample sizes often found a positive association with HDL-cholesterol and an

inverse association with triglycerides[13]. The current pooled study showed that the levels of these lipids were within physiological range in both cases and controls, and that absolute differences were small in mmol/L. However, our data suggest that an increase of HDL from the 25th percentile to the 75th percentile coincides with an AMD risk increase of about 20%. Selective survival does not appear to explain our findings, as the association was already present in the youngest age group (<= 65 yrs). The exact clinical interpretation remains to be defined. Nevertheless, the findings contribute to the understanding of AMD pathogenesis.

Animal research has provided some key insights in retinal lipid metabolism. Studies in rodents showed that most lipids in the retina are synthesized locally and up to a quarter is derived from the systemic circulation[45.] Another study in mice showed that a high fat diet increases cholesterol in the retina, but not as much as in the circulation. These results suggest that transport from the systemic circulation to the retina does take place, albeit modestly. Although LDL delivers cholesterol most efficiently from the systemic circulation to the retina, HDL-cholesterol, with ApoA-I as its major lipid component[46], does this as well via scavenger receptors[26, 47, 48]. The RPE processes the internalized lipids and subsequently secretes them again on the apical side via ABCA1 transporters into the inter-photoreceptor matrix. Thereafter, lecithin-cholesterol acyltransferase (LCAT), located at the surface of nascent HDL[49], converts free cholesterol into esterified cholesterol,[50] which are present in nascent HDL. In this way LCAT transforms nascent HDL into larger, mature HDL, while LIPC hydrolyzes phospholipids in the HDL lipoprotein[23, 51]. As suggested by Tserentsoodol *et al*[26], due to the absence of LDL in the retina, it is possible that CETP has a role in transferring esterified cholesterol between lipoproteins or photoreceptor membranes. In the inter-photoreceptor matrix, HDL functions as a transport vehicle between the RPE and the photoreceptors supporting the high synthesis and degradation of the lipid-rich photoreceptor disks[26]. The RPE maintains the lipid balance by transporting lipoproteins back to Bruchs membrane[52]. The lipid contents of these lipoproteins resemble that of LDL lipoproteins rather than HDL as it has a high abundance of esterified cholesterol, but both ApoA and ApoB[53]. It has been proposed that this large amount of esterified cholesterol acts as a barrier for lipid transport through an aging retina, thereby facilitating the formation of deposits[54]. Another mechanism proposed to form deposits is through the impairment of ABCA1 transporter of macrophages, which impairs the efflux of free cholesterol out of the macrophage. This results in senescent macrophages with high levels of cholesterol in the retina of mice[55].

Interestingly, lipoproteins appear to be closely related to the complement system, the major pathway in AMD pathogenesis. Proteomic studies have shown that HDL-lipoproteins can contain essential complement components, such as C1, C2, C3, C5 and Factor B[56-58]. One study showed that CFH and lipoproteins have competitive binding in the sub-RPE extracellular matrix, when CFH is low lipoproteins can accumulate sub-RPE[59]. By contrast, HDL can also carry complement regulators such as FH1, CFHR4 and CFHR5[60, 61]. ApoA-I attached to HDL can bind clusterin, a complement lysis inhibitor that stops the complement cascade just before the C5b-9 complex is inserted into the target[62]. These findings suggest that HDL is involved in pro-inflammatory as well as complement inhibitory tasks. Higher HDL levels may cause imbalance of the physiological homeostasis[8, 63]. Taken together, this plethora of biological leads supports the contention that HDL may play a role in the initiation of AMD. More comprehensive research into lipid metabolism in the retina is warranted.

In our study, we found elevated levels of HDL-cholesterol in the circulation and decreased levels of triglycerides in persons with AMD. In more detailed analysis, we observed a higher concentration of extra-large HDL particles with a higher total lipid and phospholipid content, which are under genetic control of CETP and LIPC. The high phospholipid content of extra-large HDL is very likely related to the larger particle size, since phospholipids compose the outer shell of the lipoprotein. CETP may exert its effect on AMD partly through systemic HDL, in line with previous Mendelian randomization studies[24, 66]. The opposing effects which we found for LIPC are less easily explained, but have been observed by others[23, 24]. This finding suggests that systemic HDL may be a biomarker rather than directly causally related to AMD. In a larger study, Kettunen *et al*[33] found more genetic effects on lipid sub fractions; variants in *CETP* and *APOE* also had a decreasing effect on the small VLDL sub fractions, while a variant in *ABCA1* increased extra-large HDL. Our smaller sample size hampered us to replicate these findings.

Where do these lipid associations fit in the chronology of AMD development? The more pronounced risk for early AMD and increasing odds ratios of HDL-cholesterol for the larger size drusen suggests that lipids play an important role at the early phase of disease. Hypothetically, intervention at this phase would be most promising in preventing blindness. We did not find statistical significance for any lipid sub fraction in only those using lipid-lowering drugs, possibly because there is no effect, but probably due to the lower power in this subgroup. Evidence from other studies indicate that statins increase HDL levels slightly[67] but reduce extra-large HDL[68], and that HDL protein composition may change as well[69]. Most epidemiologic studies do not find any effect of lipid lowering drugs on AMD[8, 70-72]; however, one study observed a slower progression of AMD in persons with a certain complement factor H risk

variant[74]. Large randomized controlled trials with long term follow-up are needed to clarify the relation between lipid lowering drugs and AMD.

In conclusion, this study showed that HDL-cholesterol and triglycerides levels are particularly associated with early AMD, mostly through the association with drusen. Extra-large HDL sub fractions seem to be drivers of this association. Whether systemic lipids directly influence lipid metabolism in the retina or whether these lipids mirror pathology in the retina is a question that remains to be answered.

FIGURE LEGENDS

Figure 1. Association of HDL-cholesterol and triglycerides with age-related macular degeneration characteristics

Figure 2. The association of metabolic variables and AMD.
Each bar represents the association with AMD, the size of the bar is the odds ratio, coloring refers to effect direction and significance. Dots indicate Bonferroni statistically significant metabolic variables corrected for age, sex and lipid lowering drugs. Labels describe the properties measured in each lipid sub fraction (*P*, concentration of particles; L, total lipids; PL, phospholipids; C, total cholesterol; CE, cholesterol esters; FC, free cholesterol; TG, triglycerides). List of abbreviations is in the annex.

Figure 3. Stage dependent relationship of the six associated lipid sub fractions with AMD.
Error bars indicate 95% confidence intervals of the mean.

Précis: (max 35 words)

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.

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Annex

Abbreviation	Name
XXL_VLDL_C	Total cholesterol in extremely large VLDL
XXL_VLDL_CE	Cholesterol esters in extremely large VLDL
XXL_VLDL_FC	Free cholesterol in extremely large VLDL
XXL_VLDL_L	Total lipids in extremely large VLDL
XXL_VLDL_P	Concentration of extremely large VLDL
XXL_VLDL_PL	Phospholipids in extremely large VLDL
XXL_VLDL_TG	Triglycerides in extremely large VLDL
XL_VLDL_C	Total cholesterol in extra-large VLDL
XL_VLDL_CE	Cholesterol esters in extra-large VLDL
XL_VLDL_FC	Free cholesterol in extra-large VLDL
XL_VLDL_L	Total lipids in extra-large VLDL

XL_VLDL_P	Concentration of extra-large VLDL
XL_VLDL_PL	Phospholipids in extra-large VLDL
XL_VLDL_TG	Triglycerides in extra-large VLDL
L_VLDL_C	Total cholesterol in large VLDL
L_VLDL_CE	Cholesterol esters in large VLDL
L_VLDL_FC	Free cholesterol in large VLDL
L_VLDL_L	Total lipids in large VLDL
L_VLDL_P	Concentration of large VLDL
L_VLDL_PL	Phospholipids in large VLDL
L_VLDL_TG	Triglycerides in large VLDL
M_VLDL_C	Total cholesterol in medium VLDL
M_VLDL_CE	Cholesterol esters in medium VLDL
M_VLDL_FC	Free cholesterol in medium VLDL
M_VLDL_L	Total lipids in medium VLDL
M_VLDL_P	Concentration of medium VLDL
M_VLDL_PL	Phospholipids in medium VLDL
M_VLDL_TG	Triglycerides in medium VLDL
S_VLDL_CE	Cholesterol esters in small VLDL
S_VLDL_L	Total lipids in small VLDL
S_VLDL_P	Concentration of small VLDL
S_VLDL_PL	Phospholipids in small VLDL
S_VLDL_TG	Triglycerides in small VLDL
XS_VLDL_C	Total cholesterol in extra-small VLDL
XS_VLDL_CE	Cholesterol esters in extra-small VLDL
XS_VLDL_FC	Free cholesterol in extra-small VLDL
XS_VLDL_L	Total lipids in extra-small VLDL
XS_VLDL_P	Concentration in extra-small VLDL
XS_VLDL_PL	Phospholipids in extra-small VLDL
XS_VLDL_TG	Triglycerides in extra-small VLDL
IDL_C	Total cholesterol in IDL
IDL_CE	Cholesterol esters in IDL
IDL_FC	Free cholesterol in IDL
IDL_L	Total lipids in IDL
IDL_P	Concentration in IDL
IDL_PL	Phospholipids in IDL
IDL_TG	Triglycerides in IDL
L_LDL_C	Total cholesterol in large LDL
L_LDL_CE	Cholesterol esters in large LDL
L_LDL_FC	Free cholesterol in large LDL
L_LDL_L	Total lipids in large LDL
L_LDL_P	Concentration in large LDL
L_LDL_PL	Phospholipids in large LDL

L_LDL_TG	Triglycerides in large LDL
M_LDL_C	Total cholesterol in medium LDL
M_LDL_CE	Cholesterol esters in medium LDL
M_LDL_FC	Free cholesterol in medium LDL
M_LDL_L	Total lipids in medium LDL
M_LDL_P	Concentration of medium LDL
M_LDL_PL	Phospholipids in medium LDL
M_LDL_TG	Triglycerides in medium LDL
S_LDL_C	Total cholesterol in small LDL
S_LDL_CE	Cholesterol esters in small LDL
S_LDL_FC	Free cholesterol in small LDL
S_LDL_L	Total lipids in small LDL
S_LDL_P	Concentration of small LDL
S_LDL_PL	Phospholipids in small LDL
S_LDL_TG	Triglycerides in small LDL
XL_HDL_C	Total cholesterol in extra-large HDL
XL_HDL_CE	Cholesterol esters in extra-large HDL
XL_HDL_FC	Free cholesterol in extra-large HDL
XL_HDL_TG	Triglycerides in extra-large HDL
L_HDL_C	Total cholesterol in large HDL
L_HDL_CE	Cholesterol esters in large HDL
L_HDL_FC	Free cholesterol in large HDL
L_HDL_L	Total lipids in large HDL
L_HDL_P	Concentration of large HDL
L_HDL_PL	Phospholipids in large HDL
L_HDL_TG	Triglycerides in large HDL
M_HDL_C	Total cholesterol in medium HDL
M_HDL_CE	Cholesterol esters in medium HDL
M_HDL_FC	Free cholesterol in medium HDL
M_HDL_L	Total lipids in medium HDL
M_HDL_P	Concentration in medium HDL
M_HDL_PL	Phospholipids in medium HDL
M_HDL_TG	Triglycerides in medium HDL
S_HDL_C	Total cholesterol in small HDL
S_HDL_CE	Cholesterol esters in small HDL
S_HDL_FC	Free cholesterol in small HDL
S_HDL_L	Total lipids in small HDL
S_HDL_P	Concentration of small HDL
S_HDL_PL	Phospholipids in small HDL
S_HDL_TG	Triglycerides in small HDL
VLDL_C	Total cholesterol in VLDL
VLDL_D	VLDL diameter
VLDL_TG	Triglycerides in VLDL

LDL_C	Total cholesterol in LDL
LDL_D	LDL diameter
LDL_TG	Triglycerides in LDL
HDL_C	Total cholesterol in HDL
HDL_D	HDL diameter
HDL_TG	Triglycerides in HDL
HDL2_C	Total cholesterol in HDL2
HDL3_C	Total cholesterol in HDL3
Serum_C	Serum total cholesterol
Serum_TG	Serum total triglycerides
TotCho	Total cholines
Remnant_C	Remnant cholesterol (non_HDL, non_LDL _cholesterol)
ApoA1	Apolipoprotein A_I
ApoB	Apolipoprotein B
EstC	Esterfied cholesterol
FreeC	Free cholesterol
FAw3	Omega 3 fatty acids
FAw3_FA	Omega 3 fatty acids to total fatty acids ratio
FAw6	Omega 6 fatty acids
FAw6_FA	Omega 6 fatty acids to total fatty acids ratio
TotFA	Total fatty acids
FALen	Fatty acid length
UnsatDeg	Estimated degree of unsaturation
CLA	Conjugated linoleic acid
LA	Linoleic acid
MUFA	Monounsaturated fatty acids
PUFA	Polyunsaturated fatty acids
SFA	Saturated fatty acids
DHA	Docosahexaenoic acid
TG_PG	Triglycerides to phosphoglycerides ratio
CLA_FA	Conjugated linoleic acid to total fatty acids ratio
DHA_FA	Docosahexaenoic acid to total fatty acids ratio
LA_FA	Linoleic acid to total fatty acids ratio
MUFA_FA	Monounsaturated fatty acids to total fatty acids ratio
PUFA_FA	Polyunsaturated fatty acids to total fatty acids ratio
SFA_FA	Saturated fatty acids to total fatty acids ratio
DAG	Diacylglycerol
DAG_TG	Diacylglycerol to triglycerides ratio
TotPG	Total phosphoglycerides
PC	Phosphatidylcholine and other cholines
SM	Sphingomyelins
AcAce	Acetoacetate
Ace	Acetate

Ala	Alanine
Alb	Albumin
bOHBut	3_hydroxybutyrate
Cit	Citrate
Crea	Creatinine
Glc	Glucose
Gln	Glutamine
Gp	Glycoprotein acetyls
His	Histidine
Ile	Isoleucine
Lac	Lactate
Leu	Leucine
Phe	Phenylalanine
Pyr	Pyruvate
Tyr	Tyrosine
Val	Valine

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Table 1. Descriptive statistics in Rotterdam Study I, visit 4.

Descriptive statistics	Controls N=1066	Cases N=464	p-value	OR	95% CI
Sex, % female	55.5% (N=592)	57.5% (N=267)	0.83	1.03	0.82 - 1.29
Age (years)	74.7 (SD 5.9)	78.1 (SD 6.6)	**<0.0001**	1.09	1.07 - 1.11
BMI	27.5 (SD 3.9)	27.0 (SD 3.9)	0.16	0.98	0.95 – 1.01
Smoking					
Former %	57.3 % (N=598)	54.3% (N=248)	0.69	0.94	0.71 – 1.25
Current %	14.5% (N=151)	15.5% (N=71)	0.26	1.24	0.85 - 1.81
Hypertension %	85.7% (N=911)	88.4% (N=410)	0.83	0.96	0.68 – 1.36
Diabetes	15.4% (N=152)	15.6% (N=68)	0.86	0.97	0.70 – 1.34
Lipid lowering drugs	24.2% (N=257)	17.0% (N=79)	**0.02**	0.72	0.54 – 0.96

Corrected for age and sex

Table 2. Baseline data and results of logistic regression analysis of the fourteen European studies.

		Controls *N*=23782	Cases *N*=7171	*p*-value	OR	95% CI
AMD						
Early AMD			*N* = 4730			
Late AMD			*N* = 2441			
Sex, % female	Early		61.7% (N=2918)	**<0.0001**	1.21	1.13 - 1.29
	Late		59.4% (N=1449)	0.74	0.98	0.88 – 1.10
	Any	57.5% (*N*=13680)	60.9% (*N*=4367)	**<0.0001**	1.15	1.09 - 1.23
Age (years)	Early		72.7 (SD 8.4)	**<0.0001**	1.06	1.06 – 1.06
	Late		76.9 (SD 8.1)	**<0.0001**	1.12	1.11 – 1.13
	Any	68.1 (SD 8.7)	74.1 (SD 8.5)	**<0.0001**	1.08	1.07 - 1.08
BMI	Early		26.6 (SD 4.2)	**0.008**	0.99	0.98 – 1.00
	Late		26.4 (SD 4.0)	**<0.0001**	1.03	1.02 – 1.05
	Any	27.0 (SD 4.3)	26.5 (SD 4.1)	0.543	1.00	0.99 – 1.05
Smoking	Early					
Former %			40.4% (N=1843)	0.77	1.01	0.94 – 1.09
Current %			8.7% (N=399)	0.14	1.10	0.97 – 1.24
	Late					
Former %			44.8% (N=917)	**<0.0001**	1.51	1.31 - 1.75
Current %			12.3% (N=253)	**<0.0001**	3.29	2.66 – 4.07
	Any					
Former %		41.3% (*N*=9530)	41.7% (*N*=2760)	**0.02**	1.09	1.01 - 1.17
Current %		12.8% (*N*=2947)	9.9% (*N*=652)	**<0.0001**	1.37	1.22 - 1.53
Hypertension %	Early		48.7% (N=2153)	0.30	1.04	0.97– 1.12
	Late		45.3% (N=977)	0.84	1.01	0.90 – 1.14
	Any	49.0% (*N*=11010)	47.6% (*N*=3130)	0.43	1.03	0.96 - 1.10
Diabetes	Early		9.7% (N=435)	0.35	0.95	0.84 – 1.06
	Late		13.2% (N=284)	**0.002**	1.33	1.11 – 1.58
	Any	10.7% (*N*=2408)	10.8% (*N*=719)	0.70	1.02	0.92 - 1.13
Lipid lowering drugs	Early		24.7% (N=1084)	**0.006**	0.89	0.83 – 0.97
	Late		22.5% (N=459)	0.86	0.99	0.85 – 1.14
	Any	24.5% (*N*=5492)	24.0% (*N*=1543)	**0.004**	0.90	0.83 - 0.97

Odds ratios are corrected for age, sex and study site. AMD = age-related macular degeneration, BMI= body mass index, OR = odds ratio

Table 3. Mixed-effects logistic regression associations of blood lipids with age-related macular degeneration

Lipid		Controls Median (25th and 75th percentile)	Cases Median (25th and 75th percentile)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.90-6.30) N=20555	5.58 (4.80-6.30) N=3907	0.98	0.95-1.01	0.24
	Late AMD	5.60 (4.90-6.30) N=20234	5.66 (4.90-6.50) N=1620	1.03	0.997-1.07	0.07
	Any AMD	5.60 (4.90-6.30) N=20555	5.60 (4.80-6.34) N=5538	1.00	0.97-1.02	0.67
HDL-cholesterol	Early AMD	1.40 (1.16-1.69) N=19931	1.50 (1.23-1.80) N=3802	1.34	1.22-1.48	**6.48×10^{-10}**
	Late AMD	1.40 (1.16-1.69) N=19662	1.47 (1.20-1.79) N=1626	1.12	1.002-1.24	0.044
	Any AMD	1.40 (1.16–1.69) N=19931	1.50 (1.22-1.80) N=5439	1.21	1.14-1.29	**1.35×10^{-9}**
LDL-cholesterol	Early AMD	3.49 (2.84-4.14) N=19590	3.37 (2.71-4.02) N=3746	0.96	0.92-0.999	0.045
	Late AMD	3.49 (2.85-4.14) N=19334	3.45 (2.79-4.14) N=1580	1.01	0.97-1.06	0.51
	Any AMD	3.49 (2.84-4.14) N=19590	3.39 (2.74-4.07) N=5337	0.98	0.95-1.01	0.13
Triglycerides	Early AMD	1.34 (1.00-1.87) N=19539	1.30 (0.97-1.80) N=3768	0.88	0.84-0.92	**2.44×10^{-7}**
	Late AMD	1.34 (1.00-1.87) N=19474	1.43 (1.01-2.01) N=1601	1.01	0.97-1.06	0.57
	Any AMD	1.34 (1.00-1.87) N=19539	1.32 (0.98-1.86) N=5374	0.94	0.91-0.97	**2.35×10^{-5}**

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 4. Mixed-effects logistic regression for any age-related macular degeneration with interaction terms with fasting vs. non-fasting

Interaction term	OR	95% CI	p-value
Total Cholesterol *fasting/non-fasting	1.00	0.96 – 1.04	0.94
HDL-cholesterol*fasting/non-fasting	0.85	0.76 – 0.95	**0.0054**
LDL-cholesterol*fasting/non-fasting	1.01	0.96 – 1.06	0.77
Triglycerides *fasting/non-fasting	1.06	1.004- 1.13	**0.038**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site, plasma or serum and fasting state. Non-fasting = 1, fasting =0.

Table 5. Mixed-effects logistic regression for any age-related macular degeneration in fasting samples

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.60 (1.40) N=17122	5.50 (1.40) N=3096	0.99	0.96 – 1.01	0.28
HDL-cholesterol	1.38 (0.51) N=16498	1.50 (0.58) N=2979	1.25	1.16 – 1.34	**4.67×10^{-9}**
LDL-cholesterol	3.48 (1.28) N=16184	3.37 (1.29) N=2933	0.97	0.94 – 0.998	0.04
Triglycerides	1.32 (0.86) N=16106	1.23 (0.79) N=2942	0.93	0.89-0.96	**3.63×10^{-5}**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 6. Mixed-effects logistic regression for any age-related macular degeneration in non-fasting samples

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.70 (1.50) N=3433	5.70 (1.60) N=2442	1.03	0.97 – 1.08	0.34
HDL-cholesterol	1.50 (0.58) N=3433	1.49 (0.56) N=2460	1.21	1.06 – 1.40	**0.006**
LDL-cholesterol	3.50 (1.38) N=3406	3.40 (1.37) N=2404	1.01	0.95 – 1.07	0.74
Triglycerides	1.43 (0.95) N=3433	1.49 (0.97) N=2432	0.95	0.89 – 1.01	0.11

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, study site and plasma or serum.

Table 7. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for plasma or serum samples

Interaction term	OR	95% CI	p-value
Total Cholesterol * plasma serum	0.97	0.90 – 1.04	0.43
HDL-cholesterol *plasma serum	1.03	0.85 – 1.25	0.77
LDL-cholesterol *plasma serum	0.98	0.89 – 1.06	0.56
Triglycerides *plasma serum	0.90	0.81- 1.01	**0.044**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. Plasma serum is coded as; plasma = 0, serum =1.

Table 8. Mixed-effects logistic regression associations of **PLASMA** lipids with age-related macular degeneration

Lipid		Controls Median (IQR)	Cases Median (IQR)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.68 (5.03-6.38) N=2769	5.75 (5.07-6.46) N=359	1.05	0.94-1.17	0.393
	Late AMD	5.71 (5.06-6.40) N=2539	5.66 (5.03-6.31) N=348	1.00	0.95-1.06	0.88
	Any AMD	5.68 (5.03-6.38) N=2769	5.69 (5.06-6.38) N=713	1.01	0.97-1.05	0.53
HDL-cholesterol	Early AMD	1.42 (1.17-1.68) N=2711	1.50 (1.24-1.80) N=358	1.26	0.92-1.71	0.133
	Late AMD	1.41 (1.17-1.68) N=2532	1.68 (1.33-2.07) N=347	1.09	0.91-1.29	0.35
	Any AMD	1.42 (1.17-1.68) N=2711	1.57 (1.28-1.93) N=711	1.09	0.97-1.21	0.15
LDL-cholesterol	Early AMD	3.69 (3.08-4.30) N=2687	3.62 (3.04-4.36) N=355	1.01	0.90-1.14	0.814
	Late AMD	3.71 (3.11-4.31) N=2520	3.64 (3.09-4.31) N=347	0.98	0.91-1.05	0.55
	Any AMD	3.69 (3.08-4.30) N=2687	3.63 (3.07-4.34) N=708	1.00	0.96-1.04	0.96
Triglycerides	Early AMD	1.07 (0.79-1.48) N=2546	1.03 (0.78-1.35) N=336	1.04	0.87-1.21	0.671
	Late AMD	1.07 (0.79-1.48) N=2539	1.01 (0.75-1.41) N=347	1.04	0.95-1.09	0.30
	Any AMD	1.07 (0.79-1.48) N=2546	1.02 (0.76-1.39) N=683	1.02	0.97-1.07	0.41

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 9. Mixed-effects logistic regression associations of **SERUM** lipids with age-related macular degeneration

Lipid		Controls Median (IQR)	Cases Median (IQR)	OR for 1 mmol/L increase	95% CI	p-value
Total Cholesterol	Early AMD	5.60 (4.88-6.30) N=17786	5.53 (4.80-6.30) N=3548	0.97	0.93-1.01	0.09
	Late AMD	5.60 (4.88-6.30) N=17695	5.66 (4.80-6.50) N=1272	1.07	1.00-1.14	0.03
	Any AMD	5.60 (4.88-6.30) N=17786	5.60 (4.80-6.31) N=4825	0.99	0.95-1.02	0.43
HDL-cholesterol	Early AMD	1.40 (1.16-1.69) N=17220	1.50 (1.23-1.80) N=3444	1.36	1.23-1.50	**1.11×10^{-9}**
	Late AMD	1.40 (1.16-1.69) N=17130	1.42 (1.19-1.70) N=1279	1.19	0.99-1.42	0.06
	Any AMD	1.40 (1.16-1.69) N=17220	1.48 (1.22-1.78) N=4728	1.31	1.20-1.44	**4.12×10^{-9}**
LDL-cholesterol	Early AMD	3.45 (2.80-4.11) N=16903	3.34 (2.69-3.98) N=3391	0.95	0.91-0.99	0.014
	Late AMD	3.45 (2.80-4.11) N=16814	3.39 (2.74-4.11) N=1233	1.04	0.97-1.12	0.26
	Any AMD	3.45 (2.80-4.11) N=16903	3.35 (2.70-4.02) N=4629	0.96	0.93-1.003	0.07
Triglycerides	Early AMD	1.40 (1.02-1.91) N=16993	1.32 (1.00-1.80) N=3432	0.87	0.83-0.91	**4.49×10^{-8}**
	Late AMD	1.40 (1.02-1.92) N=16925	1.56 (1.12-2.17) N=1254	1.01	0.94-1.09	0.72
	Any AMD	1.40 (1.02-1.91) N=16993	1.40 (1.00-1.90) N=4691	0.90	0.86-0.94	**5.07×10^{-6}**

Odds ratio estimates and 95% confidence intervals of lipid on early, late or any AMD after adjusting for age, sex, lipid lowering drug usage, body mass index, smoking, plasma or serum, fasting state and study site. Late AMD was also corrected for diabetes.
IQR = inter quartile range. p=0.0042 is Bonferroni significant.

Table 10. Mixed-effects logistic regression for any age-related macular degeneration GLMM with interaction terms for gender

Interaction terms	OR	95% CI	p-value
Total Cholesterol *gender	0.96	0.92-1.01	0.11
HDL-cholesterol * gender	1.08	0.95-1.22	0.24
LDL-cholesterol * gender	0.95	0.91-1.001	0.053
Triglycerides * gender	0.96	0.91-1.02	0.19

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum. gender is coded: 0= male, 1 = female.

Table 11. Mixed-effects logistic regression for any age-related macular degeneration for participants aged ≤ 65

Lipid	Controls Median (IQR)	Cases Late AMD Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.62 (1.36) N=8606	5.60 (1.48) N=955	0.96	0.91 – 1.01	0.09
HDL-cholesterol	1.39 (0.53) N=8310	1.48 (0.59) N=924	1.19	1.02– 1.38	**0.02**
LDL-cholesterol	3.50 (1.27) N=8188	3.40 (1.30) N=907	0.93	0.87 – 0.99	**0.02**
Triglycerides	1.34 (0.91) N=8139	1.34 (0.88) N=918	0.95	0.89- 1.02	0.15

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 12. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 65 & ≤ 80

Lipid	Controls Median (IQR)	Cases Late AMD Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.59 (1.44) N=10030	5.60 (1.51) N=3258	1.01	0.98– 1.04	0.60
HDL-cholesterol	1.40 (0.52) N=9775	1.50 (0.56) N=3199	1.25	1.15– 1.36	**1.62×10^{-07}**
LDL-cholesterol	3.48 (1.33) N=9587	3.40 (1.34) N=3146	1.00	0.96 – 1.03	0.80
Triglycerides	1.37 (0.90) N=9570	1.35 (0.89) N=3160	0.92	0.89- 0.96	**6.22×10^{-05}**

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 13. Mixed-effects logistic regression for any age-related macular degeneration for participants aged > 80

Lipid	Controls Median (IQR)	Cases Median (IQR)	OR	95% CI	p-value
Total Cholesterol	5.56 (1.45) N=1919	5.50 (1.59) N=1325	1.00	0.94 – 1.06	0.93
HDL-cholesterol	1.44 (0.56) N=1846	1.47 (0.55) N=1316	1.24	1.04 – 1.47	**0.02**
LDL-cholesterol	3.43 (1.30) N=1815	3.38 (1.34) N=1284	0.98	0.91 – 1.05	0.62
Triglycerides	1.24 (0.76) N=1830	1.30 (0.84) N=1296	0.96	0.87-1.06	0.41

Corrected for age, sex, lipid lowering drug usage, BMI, smoking, fasting state, study site and plasma or serum.

Table 14. Univariable logistic regression for AMD for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value

Lipid sub fraction	p-value	OR	95%CI
Concentration extra-large HDL	**4.06×10^{-4}**	1.24	1.10-1.40
Phospholipids in extra-large HDL	**4.32×10^{-4}**	1.24	1.10-1.40
Total cholesterol in small VLDL	**7.36×10^{-4}**	0.81	0.72-0.92
Ratio ApoB ApoA1	**7.65×10^{-4}**	0.82	0.730.92
Total lipids in extra-large HDL	**7.77×10^{-4}**	1.23	1.10-1.39
Free cholesterol in small VLDL	**7.80×10^{-4}**	0.81	0.72-0.92
Total cholesterol in extra-large HDL	1.40×10^{-3}	1.22	1.08-1.37
Cholesterol esters in extra-large HDL	1.50×10^{-3}	1.22	1.08-1.37
Cholesterol esters in small VLDL	1.56×10^{-3}	0.82	0.73-0.93
Total cholesterol in VLDL	1.74×10^{-3}	0.82	0.73-0.93

Bold p-values are Bonferroni significant, OR is for 1-SD. Lowest 10 p-values are shown.

Table 15. Association of **LDL** with age-related macular degeneration characteristics

AMD characteristic	Not present LDL Mean (+/-SD)	Present LDL Mean (+/-SD)	OR	95% CI	p-value
Small drusen <63um	3.25 (0.97) N=7079	3.59 (0.93) N=3295	0.99	0.93-1.05	0.78
Intermediate drusen >63um and <125um	3.55 (0.94) N=5651	3.62 (0.95) N=3254	0.95	0.90-1.00	0.07
Large drusen >125um	3.44 (0.98) N=11883	3.43 (0.99) N= 2066	0.99	0.94-1.05	0.74
Hyper pigmentation	3.59 (0.95) N=12982	3.48 (0.94) N=1578	0.95	0.88-1.01	0.09
Hypo pigmentation	3.58 (0.94) N=13027	3.52 (0.95) N=1531	0.98	0.92-1.05	0.56

Corrected for age, sex, lipid lowering drugs and study site

Table 16. Association of **total cholesterol** with age-related macular degeneration characteristics

AMD characteristic	Not present Total cholesterol Mean (+/-SD)	Present Total cholesterol Mean (+/-SD)	OR	95% CI	p-value
Small drusen <63um	5.40 (1.09) N=7890	5.73 (1.01) N=3450	0.99	0.94-1.04	0.67
Intermediate drusen >63um and <125um	5.56 (1.03) N=6408	5.72 (1.04) N=3412	0.97	0.93-1.02	0.24
Large drusen >125um	5.55 (1.08) N=12733	5.58 (1.08) N= 2205	1.02	0.98-1.07	0.32
Hyper pigmentation	5.63 (1.04) N=13950	5.61 (1.04) N=1646	0.97	0.91-1.02	0.24
Hypo pigmentation	5.63 (1.04) N=14004	5.64 (1.06) N=1590	1.00	0.95-1.06	0.98

Corrected for age, sex, lipid lowering drugs and study site

Table 17. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only participants using lipid lowering drugs.

Variable	p-value	OR	95%CI
Pyruvate(mmol/l)	0.005835	0.66	0.49-0.8
Albumin(signal area)	0.00821	0.69	0.52-0.90
Alanine(mmol/l)	0.013313	0.70	0.52-0.92
Tyrosine(mmol/l)	0.04998	0.76	0.57-0.99
Total lipids in small HDL	0.058837	0.76	0.57-1.01
Concentration of small HDL particles	0.060846	0.76	0.57-1.01
Diacylglycerol(mmol/l)	0.126999	0.80	0.60-1.05
Lactate(mmol/l)	0.136759	0.81	0.61-1.06
Triglycerides in extra large HDL	0.139265	1.22	0.94-1.60
Total cholesterol in small HDL	0.153029	0.82	0.62-1.08

Table 18. Univariable logistic regression for each lipid sub fraction, corrected for age, sex and lipid lowering drugs, sorted on p-value only in participants not using lipid lowing drugs

Variable	p-value	OR	95%CI
Total cholesterol in small VLDL	**0.000806**	0.796	0.695-0.908
ApoB - ApoA1 Ratio	**0.000808**	0.802	0.705-0.912
Phospholipids in extra-large HDL	**0.001003**	1.246	1.093-1.422
Concentration of extra- large HDL	0.001074	1.243	1.091-1.416
Total lipids in extra small VLDL	0.00109	0.801	0.700-0.914
Free cholesterol in small VLDL	0.001098	0.802	0.701-0.914
Total cholesterol in extra small VLDL	0.001122	0.803	0.703-0.915
Concentration of extra small VLDL	0.001232	0.803	0.702-0.916
Cholesterol esters in small VLDL	0.001387	0.804	0.702-0.918
Total cholesterol in VLDL	0.001516	0.806	0.704-0.919

Table 19 Univariable analysis of the genetic risk score for each routine lipid measurement. .

Lipid sub fractions	Estimate per 1 SD	Std. Error	p-value
Total cholesterol	-0.019	0.015	0.188
HDL-cholesterol	0.012	0.005	**0.031**
LDL-cholesterol	-0.025	0.013	0.056
Triglycerides	-0.011	0.012	0.365

AMD_Risk_score = (CFH_rs10922109 * 0.673344553) + (CFH_rs570618 * 0.553885113) + (CFH_rs148553336 * 1.171182982) + (CFH_rs187328863 * 0.385262401) + (CFH_rs35292876 * 0.431782416) + (CFH_rs191281603 * 0.891598119) + (COL4A3_rs11884770 * 0.083381609) + (ADAMTS9_AS2_rs62247658 * 0.131028262) + (COL8A1_rs140647181 * 0.615185639) + (COL8A1_rs55975637 * 0.148420005) + (CFI_rs10033900 * 0.139761942) + (C9_rs62358361 * 0.512823626) + (C2_CFB_SKIV2L_rs181705462 * 0.444685821) + (C2_CFB_rs943080 * 0.139262067) + (KMT2E_SRPK2_rs1142 * 0.131028262) + (PILRB_PILRA_rs7803454 * 0.139761942) + (MIR6130_RORB_rs10781182 * 0.104360015) + (TGFBR1_rs1626340 * 0.127833372) + (ARHGAP21_rs12357257 * 0.113328685) + (B3GALTL_rs9564692 * 0.105360516) + (RAD51B_rs61985136 * 0.127833372) + (RAD51B_rs2842339 * 0.165514438) + (LIPC_rs2043085 * 0.139761942) + (C2_CFB_SKIV2L_rs144629244 * 1.026041596) + (LIPC_rs2070895 * 0.15082289) + (CETP_rs17231506 * 0.104360015) + (TMEM97_VTN_rs11080055 * 0.083381609) + (APOE_rs429358 * 0.400477567) + (APOE_rs73036519 * 0.094310679) + (SYN3_TIMP3_rs5754227 * 0.235722334) + (SLC16A8_rs8135665 * 0.131028262) + (ABCA1_rs2740488 * 0.116533816) + (ARMS2_HTRA1_rs3750846 * 1.075002423).

The risk score is standardized and estimates are corrected for age, sex, lipid lowering drugs, serum or plasma, fasting state and study site.

Table 20A. Mixed-effects linear regression model estimating the effect of SNPs on routine lipid measurements.

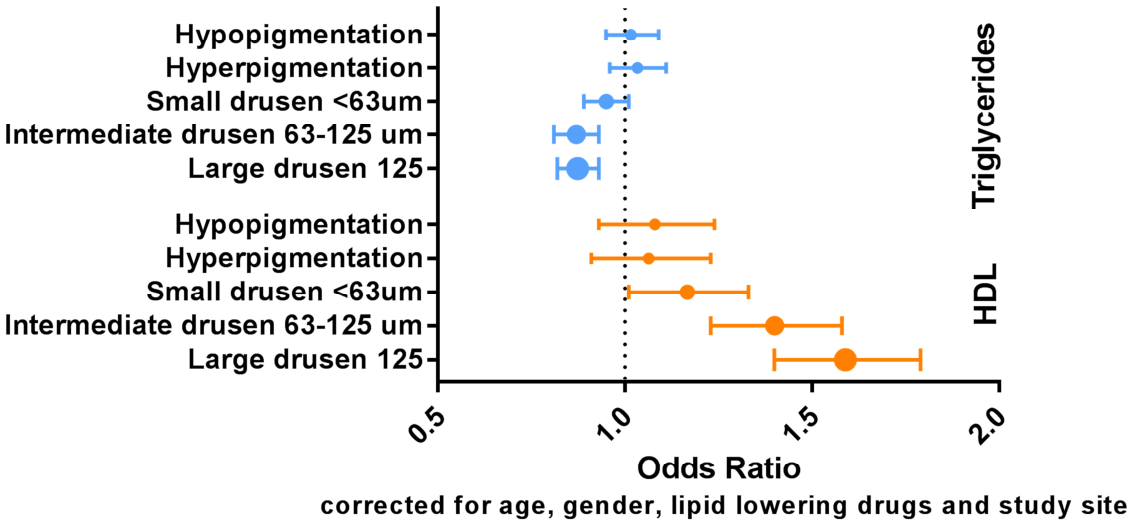
Lipid	*CETP* rs17231506 Risk allele T, reference allele C	*LIPC* rs2043085 Risk allele C, reference allele T	*LIPC* rs2070895 Risk allele G, reference allele A	*APOE* rs429358 Risk allele T, reference allele C	*APOE* rs73036519 Risk allele G, reference allele C	*ABCA1* rs2740488 Risk allele A, reference allele C
Total cholesterol	0.03 (p=0.07)	-0.019 (p=0.16)	-0.04 (p=0.008)	**-0.18 (p< 0.0001)**	0.01 (p=0.55)	**0.06 (p=0.0002)**
HDL-cholesterol	**0.08 (p< 0.0001)**	**-0.04 (p< 0.0001)**	**-0.05 (p< 0.0001)**	**0.03 (p< 0.0001)**	-0.007 (p=0.24)	**0.03 (p=0.0001)**
LDL-cholesterol	**-0.05 (p=0.0001)**	0.02 (p=0.09)	0.004 (p=0.77)	**-0.19 (p< 0.0001)**	0.01 (p=0.67)	0.04 (p=0.01)
Triglycerides	-0.025 (p=0.04)	0.001 (p=0.93)	-0.006 (p=0.66)	**-0.06 (p=0.0004)**	0.03 (p=0.03)	0.004 (p=0.78)

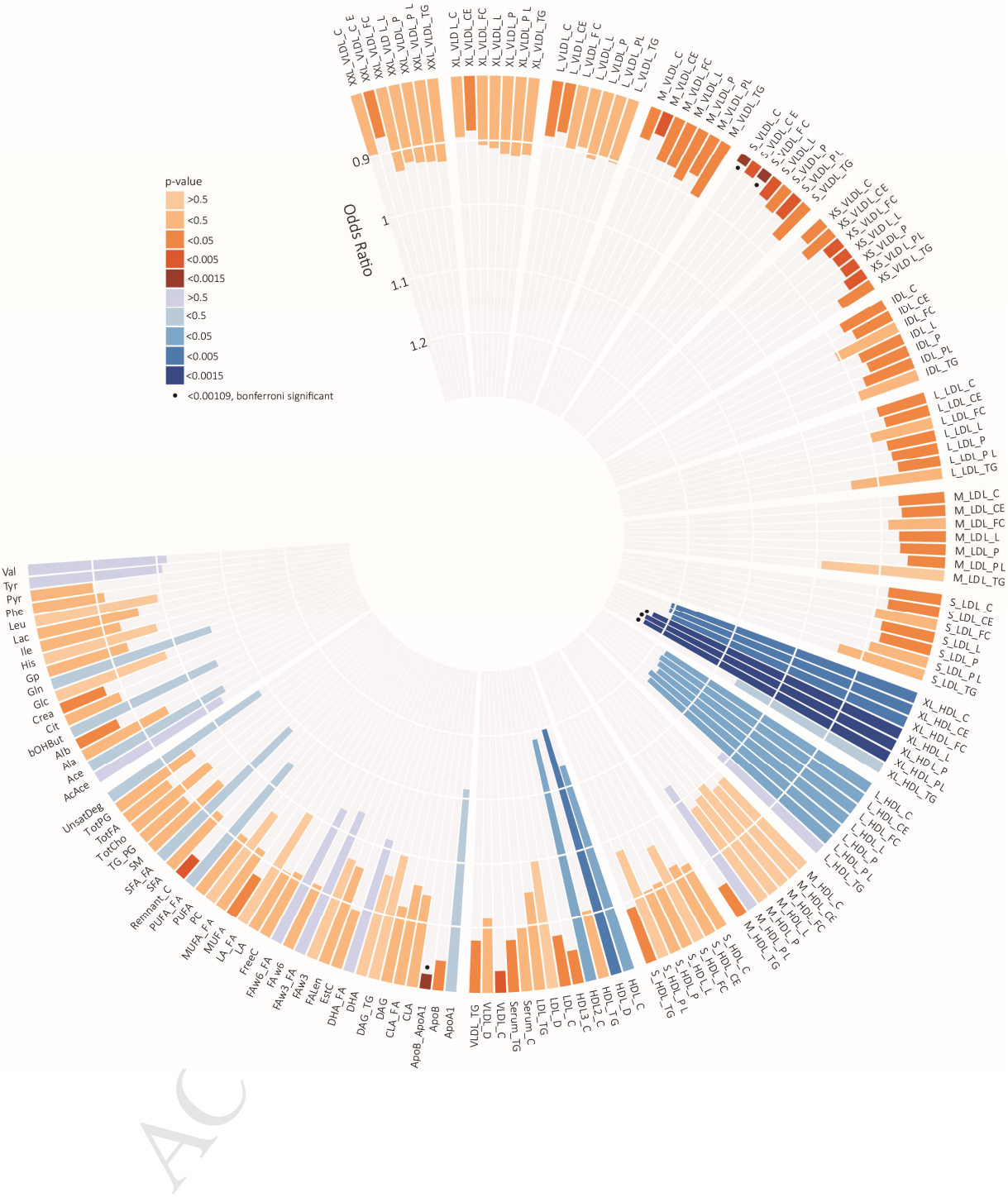
Betas are corrected for age, gender, lipid lowering drugs, plasma or serum, fasting state and study site. Betas indicate the effect of the risk allele versus the reference allele. Bonferroni: 0.05/60 = 0.00083 (8.3x10-4). Red is negative effect size, blue is positive effect size.

Table 20B. Linear regression model estimating the effect of SNPs on lipid sub fraction.

Lipid	*CETP* rs17231506 Risk allele T, reference allele C	*LIPC* rs2043085 Risk allele C, reference allele T	*LIPC* rs2070895 Risk allele G, reference allele A	*APOE* rs429358 Risk allele T, reference allele C	*APOE* rs73036519 Risk allele G, reference allele C	*ABCA1* rs2740488 Risk allele A, reference allele C
Percentage extra-large HDL	**0.15 (p= $7.68x10^{-7}$)**	**-0.14 (p= $1.00x10^{-6}$)**	**-0.13 (p= $1.55x10^{-4}$)**	-0.07 (p= 0.112)	0.04 (p=0.167)	0.05 (p=0.164)
Phospholipids in extra-large HDL	**0.15 (p= $3.51x19^{-7}$)**	**-0.13 (p= $4.0x10^{-6}$)**	**-0.13 (p= $1.04x10^{-4}$)**	-0.03 (p= 0.479)	0.04 (p=0.233)	0.03 (p=0.349)
Total cholesterol in small VLDL	-0.09 (p=0.003)	-0.09 (p=0.003)	-0.08 (p=0.02)	-0.05 (p=0.294)	-0.02 (p=0.631)	0.08 (p=0.027)
Ratio ApoB ApoA1	-0.10 (p=0.002)	0.004 (p=0.897)	-0.003 (p=0.936)	-0.09 (p=0.037)	-0.04 (p=0.250)	0.02 (p=0.521)
Total lipids in extra-large HDL	**0.15 (p=$4.06x10^{-7}$)**	**-0.14 (p=$8.59x10^{-7}$)**	**-0.12 (p=$3.13x10^{-4}$)**	-0.08 (p=0.056)	0.04 (p=0.213)	0.05 (p=0.138)
Free cholesterol in small VLDL	-0.08 (p=0.01)	-0.08 (p=0.005)	-0.09 (0.009)	0.01 (p=0.784)	-0.03 (p=0.434)	0.07 (p=0.058)

Betas are corrected for age, gender, lipid lowering drug usage, sub fractions are log+1 and standardized. Betas indicate the effect of the risk allele versus the reference allele.





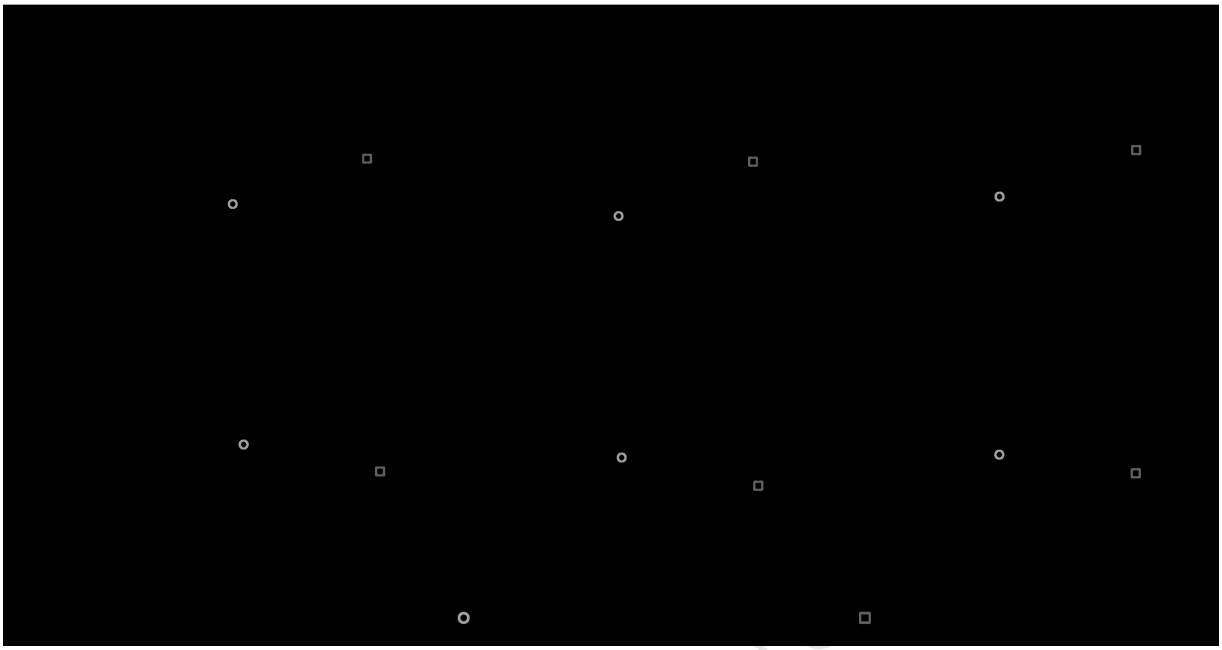
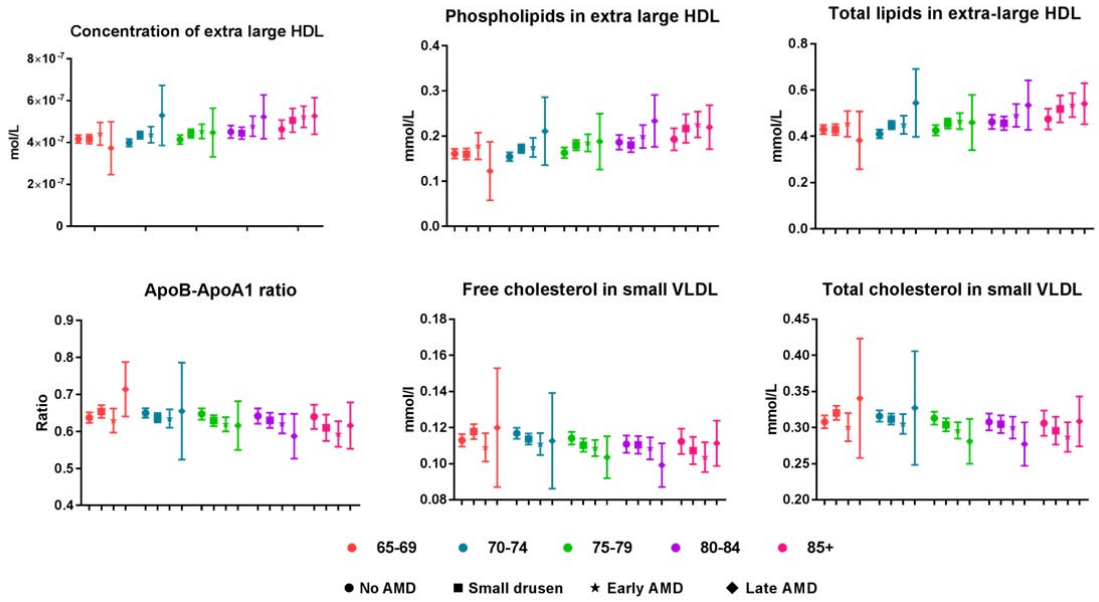


Figure 4. Dose dependent relationship of the six associated lipid sub fractions with AMD per age category. Error bars indicate 95% confidence intervals of the mean.



Précis:

HDL-cholesterol is positively associated with AMD and triglycerides negatively. This is most prominently seen in drusen as early AMD features. This association seems to be driven by larger HDL sub fractions and HDL related genetics.