Clinical and Epidemiologic Research

# Metabolite and Lipid Biomarkers Associated With Intraocular Pressure and Inner Retinal Morphology: $^{1}$H NMR Spectroscopy Results From the UK Biobank

Louis R. Pasquale,[1] Anthony P. Khawaja,[2] Janey L. Wiggs,[3] Jihye Kim,[4] Pirro Hysi,[5,6] Tobias Elze,[7] Jessica Lasky-Su,[8] Jae H. Kang,[8] and Oana Zeleznik[8]; for the UK Biobank Eye and Vision Consortium

[1]Department of Ophthalmology, Icahn School of Medicine at Mount Sinai, New York, New York, United States
[2]NIHR Biomedical Research Centre at Moorfields Eye Hospital & UCL Institute of Ophthalmology, London, United Kingdom
[3]Department of Ophthalmology, Massachusetts Eye and Ear, Harvard Medical School, Boston, Massachusetts, United States
[4]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, United States
[5]Department of Ophthalmology, King's College London, St. Thomas' Hospital, London, United Kingdom
[6]Department of Twin Research & Genetic Epidemiology, King's College London, St. Thomas' Hospital, London, United Kingdom
[7]Department of Ophthalmology, Schepens Research Eye Institute of Massachusetts Eye and Ear, Harvard Medical School, Boston, Massachusetts, United States
[8]Channing Division of Network Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, United States

Correspondence: Louis R. Pasquale, Department of Ophthalmology, Icahn School of Medicine, One Gustave L. Levy Place, Box 1183, New York, 10029 NY, USA; louis.pasquale@mssm.edu.

JHK and OZ contributed equally to this work.

A list of the UK Biobank Eye and Vision Consortium members is available at https://www.ukbiobankeyeconsortium.org.uk/people.

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**Purpose.** The purpose of this study was to assess metabolites associated with intraocular pressure (IOP) and inner retina structure.

**Methods.** We cross-sectionally assessed 168 non-fasting plasma metabolites measured by nuclear magnetic resonance (NMR) spectroscopy with IOP ($n$ = 28,195), macular retinal nerve fiber layer thickness (mRNFL; $n$ = 10,584), and macular ganglion cell inner plexiform layer thickness (mGCIPL; $n$ = 10,554) in the UK Biobank. We used multiple linear regression models adjusting for various covariates with probit-transformed metabolite levels as predictors for each outcome. Each estimate represents the difference in outcome variable per standard deviation increase in the probit-transformed metabolite values. We used the number of effective (NEF) tests and false discovery rate (FDR) to adjust for multiple comparisons for metabolites and metabolite classes, respectively.

**Results.** In individual metabolite analysis, multiple amino acids, especially branched-chain amino acids, were associated with lower IOP (−0.12 mm Hg; 95% confidence interval = −0.16 to −0.07; NEF = 2.7E-05). Albumin, 3 hydroxybutyrate, lactate, and several lipids were associated with higher IOP (range = 0.07 to 0.18 mm Hg, NEF = ≤ 0.039). In IOP-adjusted analyses, five HDL-related metabolites were associated with thinner mRNFL (−0.15 microns for all metabolites, NEF = ≤ 0.027), whereas five LDL-related metabolites were associated with thicker mGCIPL (range = 0.17 to 0.20 microns; NEF = ≤ 0.044). In metabolite class analysis, the lipid components of lipoproteins (cholesterol, triglycerides, etc.) were not associated with our outcomes (FDR > 0.2 for all); yet multiple lipoproteins were significantly (FDR < 0.05) associated with all outcomes.

**Conclusions.** Branched-chain amino acids were associated with lower IOP, HDL metabolites were associated with thinner mRNFL, and LDL metabolites were associated with thicker mGCIPL.

Keywords: intraocular pressure (IOP), optical coherence tomography (OCT), metabolites

Intraocular pressure (IOP) is a risk factor for glaucoma,[1] a disease categorized by retinal ganglion cell (RGC) loss.[2] The exact reasons for the dysregulation of aqueous humor dynamics, which contribute to elevated IOP in primary open-angle glaucoma (POAG; the most common glaucoma subtype) is unknown. Furthermore, RGCs with their arborizing dendrites, cell bodies, and axons that course through the retina and optic nerve to the lateral geniculate nucleus do not turn over. Very little is known about the mechanisms of how RGCs maintain lifelong homeostatic mechanisms. Systematic investigations of metabolites related to IOP, retinal nerve fiber layer (RNFL) thickness,

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and ganglion cell inner plexiform layer (GCIPL) thickness may provide insights into mechanisms involved in glaucoma pathogenesis.

To date, only a few studies have evaluated the metabolomics of IOP or inner retinal parameter differences. The rate of aqueous humor production and egress across the outflow pathway dictate the IOP level, and aqueous humor represents a specialized distillate of serum that is high in ascorbic acid and low in proteins. A study performed on the Metabolon platform reported a high ascorbic acid plasma metabolite level associated with lower IOP.[3] A nonfasting metabolomic study using nuclear magnetic resonance (NMR) spectroscopy in a multi-ethnic Asian population found several metabolites associated with higher IOP, including higher levels of albumin, lactate, glucose, and cholesterol esters in very large high-density lipoprotein (HDL), as well as lower levels of glutamine.[4] In a subsequent candidate metabolite study in 2 United Kingdom cohorts involving 94,323 participants, plasma total cholesterol, HDL-cholesterol, and low-density lipoprotein (LDL)-cholesterol were associated with higher IOP, although inner retinal measures were not assessed.[5] In the only biomarker panel study to date on inner retina measures (but not IOP levels) among 8952 healthy subjects from Leipzig Germany, lower plasma HDL, and higher LDL were associated with thicker circumpapillary RNFL thickness,[6] a marker of glaucoma neuronal status.

The relationship between lipid metabolites and glaucoma has been studied extensively but the findings are inconsistent. A meta-analysis of 29 observational studies found that higher total cholesterol and lower HDL cholesterol, but not LDL levels, were associated with increased risk of glaucoma, although considerable study heterogeneity existed due to the inclusion of various glaucoma types and the variable control for lipid-lowering medications.[7] Finally, a study with 175 cases from an Asian population reported that higher HDL3 cholesterol (a small particle HDL lipoprotein that is approximately 8.7 nm in size) was associated with lower POAG risk, but phospholipids from very large HDL (14.3 nm particle size) were associated with higher POAG risk, whereas other HDL and LDL subtypes showed null associations.[8] Other studies found positive associations between plasma triglycerides[9,10,14] and phospholipids[11–14] with glaucoma.

Given the inconsistent findings and following up our metabolomic study on POAG,[14] we analyzed 168 nonfasting plasma metabolites measured by NMR spectroscopy in relationship to IOP (28,195 participants) and macular region inner retinal thicknesses (both RNFL and GCIPL in 10,584 and 10,554 participants, respectively) determined with ocular coherence tomography in the UK Biobank. NMR spectroscopic analysis allows for the quantification of various low molecular weight metabolites, albumin, and lipoprotein subtypes.[15]

## Methods

### Study Population – UK Biobank

From 2006 to 2010 (considered as baseline), over 500,000 subjects aged 40 to 69 years from various data acquisition centers throughout the United Kingdom contributed data to the UK Biobank repository.[16] We used baseline questionnaires and metabolomic data from the UK Biobank for this analysis (http://www.ukbiobank.ac.uk). The National Information Governance Board for Health and Social Care and the National Health Service North West Multicenter Research Ethics Committee (reference number 06/MRE08/65) approved the UK Biobank resource, and this study was completed under application number 36741.

### Metabolite Profiling – UK Biobank

Non-fasting EDTA plasma samples were archived from a random subset of 114,466 participants for planned multiomics analyses in 2007 to 2010. Plasma samples were thawed and submitted for targeted high-throughput NMR metabolomics on the Nightingale platform (Nightingale Health Ltd., Helsinki, Finland) in 2019.[17] In contrast to liquid chromatography/mass spectroscopy, in $^{1}$H NMR spectroscopy, samples are subjected to a 650 MHz magnetic field without a processing step that strips them of proteins. Molecules with H atoms yield distinctive spectral shapes with areas under their curves proportional to the molecules' concentration based on chemical shifts and J coupling split patterns determined from quantum mechanics,[15,18] allowing for detailed quantification. The platform provides quantification of 249 metabolic measures, including routine clinical lipids (37 biomarkers are certified for clinical use in the European Union), lipoprotein subclasses, and other metabolites measured in molar concentration units. Of the 249 measures, we focused on 168 measures that were concentrations of various metabolites, as the 81 remaining measures were ratios among metabolites, percentages of individual metabolites of total classes, or degree of unsaturation. These 168 metabolites belonged to 17 classes, and we evaluated the following 14 classes comprised of at least 3 metabolites: amino acids, triglycerides, lipoprotein subclasses, lipoprotein particle concentrations, lipoprotein particle sizes, phospholipids, total lipids, cholesterol, free cholesterol, cholesteryl esters, other lipids, fatty acids, glycolysis related metabolites, and ketone bodies. In an additional analysis, we also investigated 15 lipoprotein subclasses: small HDL, medium HDL, large HDL, very large HDL, small LDL, medium LDL, large LDL, IDL, very small VLDL, small VLDL, medium VLDL, large VLDL, very large VLDL, extremely large VLDL, and chylomicrons.

### Measurement of IOP in the UK Biobank

Starting in late 2009, a subset of participants underwent ocular examinations that included IOP measurements at various UK Biobank study sites.[19] We used the corneal-compensated IOP (IOPcc) measured with the Ocular Response Analyzer noncontact tonometer (Reichert Corporation, Philadelphia, PA), which is relatively independent of corneal biophysical parameters.[20] We excluded outliers in the extreme top and bottom 0.5 percentiles, participants with eye surgery or an eye infection in the prior month, and those with a history of glaucoma or glaucoma treatment of any kind as their untreated IOP level would be unknown with certainty. IOP measures for each participant represent the mean of the right and left eye unless data were available on one eye only, in which case that eye's IOP level was used. After the application of exclusion criteria, there were 28,195 participants with both IOP and plasma metabolite data available for analysis.

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### Measurement of Inner Retinal Thicknesses in the UK Biobank

A subset of UK Biobank participants underwent macula region optical coherence tomography (OCT) to image the macular retinal nerve fiber layer thickness (mRNFL) and macular ganglion cell inner plexiform layer thickness (mGCIPL) in 2009 to 2010 as part of the eye examination mentioned above.[19] Details regarding the performance of OCT, quality control measures implemented, data cleaning steps, and inner retinal biomarker data extraction have been previously described.[21] Briefly, OCT imaging was performed with the Topcon 3D OCT 1000 Mark II without pharmacologic mydriasis under scoptic conditions. Imagers used the 3-dimensional 6 × 6 $mm^2$ macular volume scan mode and segmented with the Topcon Advanced Boundary segmentation algorithm (version 1.6.1.1). Various quality control filters were applied. Images with a quality score <45, as well as artifacts secondary to blinking, saccades, and segmentation errors were excluded. Finally, images from participants with non-glaucomatous neurodegeneration were removed (Alzheimer's disease, Parkinson's disease, and multiple sclerosis). Of the 67,321 individuals that underwent macular spectral domain OCT (SD-OCT), 10,584 and 10,554 participants met inclusion criteria and had available metabolomics data in relation to mRNFL and mGCIPL, respectively.

### Statistical Analysis

In model 1, we report crude unadjusted associations and in model 2, we adjusted for time since the last meal/drink (≤4, 5–8, and 9+ hours), age, sex, race/ethnicity (Asian, Black, White, and other), season, and time of day of specimen collection. Model 3 incorporated model 2 with further adjustment for smoking status (never, past, and current smoker), number of cigarettes smoked daily among current smokers, alcohol intake frequency (daily or almost daily, 3-4 times a week, 1–2 times a week, 1–3 times a month, special occasions only, and never), coffee and tea consumption (cups per day), physical activity (metabolic equivalent of task [MET] hours/week), Townsend deprivation index (range = −6 to 11; a higher index score indicates more relative poverty for a given residential area), body mass index ($kg/m^2$), systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of coronary artery disease, systemic beta-blocker use, statin drug use, oral steroid use, and spherical equivalent refractive error (diopters). Where inner retinal biomarkers were the outcome, we performed an additional adjustment for IOPcc in analyses.

All analyses were performed with SAS version 9.4 and R version 4.0.3. Metabolite values were transformed to probit scores and used as continuous variables (per 1 standard deviation [SD] increase) to calculate *P* values. We estimated the beta coefficients (or differences in each outcome) and 95% confidence intervals (CIs) per 1 SD increase in metabolite levels.

For evaluating individual metabolites, the number of effective (NEF)[22] test was used to adjust for multiple comparisons as the NEF test accounts for the high correlation structure of the metabolomics data. An NEF test of $P < 0.05$ was considered statistically significant and an NEF test of $P < 0.2$ was considered worthy of additional analysis given that this was an exploratory study.

We used Metabolite Set Enrichment Analysis (MSEA) for metabolite class analyses.[23] In MSEA, the effect size for a metabolite class is estimated with a normalized enrichment score (NES). The NES is defined by the degree to which a set of metabolites is over-represented at the extremes (top [metabolites with positive effect estimates] or bottom [metabolites with negative effect estimates]) of the entire list of measured metabolites, ranked by their real effect estimates. The NES is adjusted to account for the size of the metabolite set. As metabolite classes overall are not correlated, we used the false discovery rate (FDR)[24] to adjust for multiple comparisons with FDR < 0.05 considered statistically significant and FDR < 0.2 considered nominally significant.

## Results

### Study Population

All participants in this study were free of glaucoma and not using any ocular hypotensive medicines based on responses to a touch screen questionnaire regarding ocular health. The mean age (SD) for participants from the parent population ($n = 189{,}706$) who completed the ocular health touch screen questionnaire was 56.8 years (SD = 8.0; Table 1). Participant demographics, lifestyle tendencies, medical status, and ocular parameters were materially similar across the datasets for the different outcomes (see Table 1). Figure 1 summarizes the degree of overlap between the datasets. For example, most participants with inner retinal biomarker measures (10,022 of 10,584 and 10,022 of 10,554 for mRNFL and mGCIPL, respectively) also had IOPcc measurements. All ophthalmic biomarker measurements were made within 2 years of plasma sample collection.

### Relation Between Individual Metabolites and IOPcc

The mean IOP (SD) for included participants was 15.9 (3.9) mm Hg (see Table 1). Higher total concentration of branched-chain amino acids (BCAAs) was associated with significantly lower IOP (−0.12 mm Hg; 95% CI = −0.16 to −0.07; NEF = 2.7 E-05; model 3, Table 2). All three BCAAs (leucine, isoleucine, and valine) were among the metabolites associated with lower IOPcc at the NEF < 0.05 level (IOP range = −0.16 to −0.08 mm Hg; NEF ≤ 0.02; see model 3, Table 2). One essential amino acid (phenylalanine), and two non-essential amino acids (tyrosine and glutamine) were also associated with lower IOPcc (range = −0.13 to −0.08 mm Hg; NEF ≤ 4.8 E-03; see model 3, Table 2).

Overall, many more metabolites were associated with higher IOPcc than with lower IOPcc. Selected top metabolites associated with higher IOPcc are also shown in Table 2. Albumin (the most abundant circulating protein in plasma), 3-hydroxybutyrate (a ketone body), lactate (a metabolic waste product), pyruvate (a product of glycolysis), and citrate (an intermediate of the tricarboxylic acid cycle) showed the most significant positive associations (range = +0.08 to +0.18 mm Hg; NEF ≤ 5.2 E −03; see model 3, Table 2). Both LDL cholesterol and HDL cholesterol were also associated with higher IOP (range = +0.09 to +0.12 mm Hg; NEF ≤ 4.9 E −03; see model 3, Table 2). Supplementary Table S1 indicates that many lipoproteins across a spectrum of size and density, as well as other metabolites, were associated with higher IOPcc at the NEF < 0.05 level in model 3. The relation between all metabolites and IOPcc, including those not showing significant associations, is provided in Supplementary Table S2.

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**Table 1.** Demographic, Medical, and Ocular Characteristics Stratified by Quartiles of Corneal Compensated Intraocular Pressure (IOPcc) in the UK Biobank

| | IOP Dataset | mRNFL Dataset | mGCIPL Dataset | No Glaucoma* |
|---|---|---|---|---|
| Sample size | 28,195 | 10,584 | 10,554 | 189,706 |
| Age, years (SD) | 56.8 (8.0) | 56.3 (8.2) | 56.3 (8.2) | 56.8 (8.0) |
| Men, *n* (%) | 12,979 (46.0) | 4941 (46.7) | 4928 (46.7) | 86,491 (45.6) |
| Ethnicity, *n* (%) | | | | |
| Asian | 933 (3.3) | 285 (2.7) | 287 (2.7) | 5468 (2.9) |
| Black | 798 (2.8) | 311 (2.9) | 313 (3.0) | 4931 (2.6) |
| Other | 673 (2.4) | 242 (2.3) | 243 (2.3) | 4754 (2.5) |
| White | 25,661 (91.4) | 9705 (92.1) | 9668 (92.0) | 173,634 (92.0) |
| Smoking status, (%) | | | | |
| Never | 15,739 (56.1) | 5802 (55.0) | 5785 (55.0) | 104,615 (55.4) |
| Past | 9639 (34.3) | 3670 (34.8) | 3667 (34.9) | 65,173 (34.5) |
| Current | 2702 (9.6) | 1070 (10.1) | 1060 (10.1) | 19,087 (10.1) |
| Physical activity, MET-hours/week (SD) | 2652 (2,669) | 2682 (2741) | 2672 (2728) | 2619 (2674) |
| Body mass index, kg/m$^2$ (SD) | 27.3 (4.5) | 27.3 (4.5) | 27.3 (4.5) | 27.4 (4.6) |
| Coffee consumption, cups/day (SD) | 1.9 (1.8) | 1.9 (1.8) | 1.9 (1.8) | 1.9 (1.8) |
| Tea consumption, cups/day (SD) | 3.1 (2.1) | 3.1 (2.1) | 3.1 (2.1) | 3.1 (2.1) |
| Systolic blood pressure, mm Hg (SD) | 137.1 (18.2) | 136.8 (18.2) | 136.8 (18.2) | 137.4 (18.5) |
| Diabetes, *n* (%) | 1666 (5.9) | 530 (5.0) | 532 (5.1) | 12,549 (6.6) |
| Coronary artery disease, n (%) | 1369 (4.9) | 480 (4.5) | 478 (4.5) | 10,145 (5.3) |
| Hemoglobin A1C, mmol/mol (SD) | 36.1 (6.5) | 36.0 (6.4) | 36.0 (6.4) | 36.3 (7.1) |
| Statin use, n (%) | 5475 (19.4) | 1903 (18.0) | 1908 (18.1) | 37,919 (20.0) |
| Oral steroid use, n (%) | 665 (2.4) | 249 (2.4) | 249 (2.4) | 5152 (2.7) |
| mRNFL thickness, microns (SD) | 29.0 (3.9) | 29.0 (3.9) | 29.0 (3.8) | 28.9 (3.8) |
| mGCIPL thickness, microns (SD) | 75.3 (5.2) | 75.3 (5.2) | 75.3 (5.2) | 75.2 (5.2) |
| Intraocular pressure, mmHg (SD) | 15.9 (3.6) | 15.7 (3.5) | 15.7 (3.5) | 15.9 (3.6) |

* No glaucoma: The subset of UK Biobank participants who did not report glaucoma and were not receiving glaucoma treatment.
Values are means ± SD or percentages and are based on those with non-missing values.
Abbreviations: IOP, intraocular pressure; mRNFL, macular region retinal nerve fiber layer; mGCIPL, macular region ganglion cell inner plexiform layer; SD, standard deviation; MET, = metabolic equivalent of task.

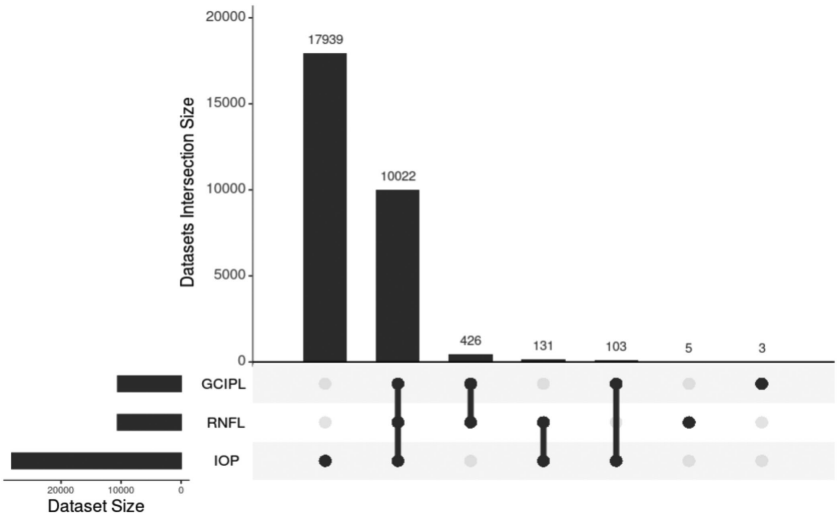


**Figure 1.** Graph summarizing the degree of overlap between the different datasets. IOP is a cornea-compensated IOP measured with the Ocular Response Analyzer. mGCIPL and mRNFL are macular region ganglion cell inner plexiform layer thickness and retinal nerve fiber layer thickness measures, respectively. Most participants with inner retinal biomarker data (10,022 out of 10,584 and 10,022 out of 10,554 in the case of mRNFL and mGCIPL, respectively) also had IOP measurements.

## Relation Between Metabolites and Inner Retinal Biomarkers

The mean mRNFL (SD) for included participants was 29.0 (3.9) microns (see Table 1). No metabolite was associated with greater mRNFL thickness. We found five metabolites associated with thinner mRNFL thicknesses at the NEF < 0.05 level: all metabolites represented various lipid components of medium HDL (−0.15 microns; NEF ≤ 0.027; model 3, Table 3). The mean mGCIPL (SD) for included participants was 75.3 (5.2) microns (see Table 1). Various lipid components of small LDL,

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**Table 2.** Top NMR Metabolites Associated With Corneal-Compensated Intraocular Pressure (IOPcc) in the UK Biobank ($N = 28,195$)*

| All Metabolites Associated With Lower IOPcc (mm Hg) | | | | | | |
|---|---|---|---|---|---|---|
| | Model 1† | | Model 2† | | Model 3† | |
| Metabolite | Beta (95% CI) | NEF | Beta (95% CI) | NEF | Beta (95% CI) | NEF |
| Isoleucine | −0.15 (−0.20 to −0.11) | 9.2 E-11 | −0.17 (−0.22 to −0.12) | 1.6 E-11 | −0.16 (−0.20 to −0.11) | 2.1 E-09 |
| Phenylalanine | −0.19 (−0.23 to −0.14) | 3.8 E-16 | −0.17 (−0.21 to −0.12) | 1.1 E-12 | −0.13 (−0.17 to −0.09) | 1.3 E-07 |
| Leucine | −0.10 (−0.14 to −0.06) | 2.6 E-04 | −0.15 (−0.20 to −0.11) | 1.4 E-09 | −0.13 (−0.18 to −0.09) | 3.4 E-07 |
| Valine | −0.03 (−0.07 to 0.02) | 0.99 | −0.08 (−0.12 to −0.03) | 0.019 | −0.08 (−0.12 to −0.03) | 0.02 |
| Total BCAAs | −0.08 (−0.12 to −0.03) | 0.021 | −0.12 (−0.17 to −0.08) | 3.2 E-06 | −0.12 (−0.16 to −0.07) | 2.7 E-05 |
| Tyrosine | −0.03 (−0.08 to 0.01) | 0.99 | −0.08 (−0.13 to −0.04) | 5.3 E-03 | −0.08 (−0.13 to −0.04) | 4.8 E-03 |
| Glutamine | −0.11 (−0.15 to −0.07) | 2.0 E-05 | −0.15 (−0.19 to −0.11) | 9.0 E-11 | −0.09 (−0.13 to −0.09) | 1.9 E-03 |
| **Selected Top Metabolites Associated With Higher IOPcc (mm Hg)** | | | | | | |
| | Model 1† | | Model 2† | | Model 3† | |
| Metabolite | Beta (95% CI) | NEF | Beta (95% CI) | NEF | Beta (95% CI) | NEF |
| Albumin | 0.16 (0.11 to 0.20) | 1.4 E-11 | 0.20 (0.16 to 0.24) | 1.3 E-19 | 0.16 (0.11 to 0.20) | 9.6 E-12 |
| 3-hydroxybutyrate | 0.25 (0.20 to 0.29) | 1.8 E-27 | 0.22 (0.17 to 0.26) | 1.7 E-20 | 0.18 (0.13 to 0.22) | 8.9 E-14 |
| Lactate | 0.14 (0.10 to 0.19) | 7.1 E-10 | 0.14 (0.10 to 0.19) | 1.0 E-08 | 0.14 (0.09 to 0.18) | 4.9 E-08 |
| Citrate | 0.13 (0.08 to 0.17) | 1.5 E-07 | 0.12 (0.08 to 0.17) | 1.1 E-06 | 0.12 (0.07 to 0.16) | 4.4 E-06 |
| Pyruvate | 0.01 (−0.04 to 0.05) | 0.99 | 0.06 (0.02 to 0.11) | 0.059 | 0.08 (0.04 to 0.12) | 5.2 E-03 |
| HDL Cholesterol | 0.03 (−0.01 to 0.08) | 0.99 | 0.20 (0.15 to 0.24) | 5.7 E-15 | 0.12 (0.06 to 0.17) | 2.9 E-04 |
| LDL Cholesterol | 0.12 (0.08 to 0.16) | 1.3 E-06 | 0.14 (0.1 to 0.18) | 3.5 E-09 | 0.09 (0.04 to 0.13) | 4.9 E-03 |

* The complete list of analytes associated with higher IOP at the NEF < 0.05 level is provided in Supplementary Table S1.

† **Model 1:** crude associations; **model 2:** adjust for time since last meal/drink (≤4, 5–8, and 9+ hours), age, sex, race/ethnicity (White, Black, and other), season, and time of day of specimen acquisition; **Model 3:** further adjustments for smoking status (never, past, and current smoker), number of cigarettes smoked daily among current smokers, alcohol intake frequency (daily or almost daily, 3–4 times a week, 1–2 times a week, 1–3 times a month, special occasions only, and never), coffee and tea consumption (cups per day), physical activity (metabolic equivalent of task (MET) hours/week), Townsend deprivation index (range = −6 to 11; a higher index score indicates more relative poverty for a given residential area), body mass index (kg/m$^2$), systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of cardiovascular disease, systemic beta-blocker use, use of statin drugs, use of oral steroids, and spherical equivalents. We accounted for multiple comparisons by using the NEF statistic.

The multiple linear regression models estimated differences in IOPcc with each one standard deviation increase in individual metabolites.

Abbreviations: IOPcc, corneal compensated intraocular pressure; CI, confidence interval; NEF, number of effective tests; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NMR, nuclear magnetic resonance.

and large LDL as well as large LDL particle concentration were associated with higher mGCIPL thickness (range = +0.18 to +0.20 microns; NEF ≤ 0.044; see Model 3, Table 3). No metabolites were associated with thinner mGCIPL thickness. The relations between all metabolites and inner retinal biomarkers are provided in Supplementary Table S2.

## Metabolite Overview

To help visualize important trends in the data, Figure 2 summarizes all metabolites in relation to the outcomes that have an association at NEF < 0.2 for at least one of the 3 outcomes in model 3. Cholesteryl esters in medium HDL were associated with higher IOPcc and thinner mRNFL at NEF < 0.05 level. Similar trends were seen for related medium HDL metabolites including cholesterol in medium HDL, free cholesterol in medium HDL, apolipoprotein A1, and medium HDL particle concentration. Several LDL-related lipoproteins were associated with higher IOP, yet some were related to nominally thicker mRNFL (for example, large LDL particle concentration), and many were associated with higher mGCIPL thickness at the NEF < 0.05 or 0.2 level. Although several BCAAs were associated with lower IOPcc, none were associated with inner retinal biomarkers.

## Metabolite Class Analysis

In the MSEA, only amino acids were associated with lower IOP in model 3 of Table 2 (NES = −3.1 mm Hg; FDR = 1.6 E-08). No other metabolite classes, were significantly associated with IOP or inner retinal biomarkers (FDR > 0.2, see Supplementary Table S2 for effect sizes and FDR values); however, among the lipoprotein subclasses, there were several significant associations (Fig. 3; exact effect sizes and FDR values are provided in Supplementary Table S2) that were consistent with those seen in individual metabolite analysis. For example, medium HDL (average diameter 10.9 nm) was associated with thinner mRNFL (NES = −2.2 microns; FDR = 1.8 E-05; see Supplementary Table S2; adjusted for model 3 covariates listed in Table 3) and mGCIPL (NES = −2.6 microns; FDR = 2.3 E-07; see Supplementary Table S2; adjusted for model 3 covariates listed in Table 3) despite a nominal association with higher IOPcc (+1.4 mm Hg; FDR = 0.16; adjusted for model 3 covariates listed in Table 2). Small LDL (average diameter 18.7 nm) was associated with thicker mRNFL (NES = +1.9 microns; FDR = 0.0098) and thicker mGCIPL (NES = +2.1 microns; FDR = 5.6 E-06; see the adjusted for model 3 covariates listed in Table 3). The relation among all metabolite classes, subclasses, and all biomarkers, including those not showing significant associations, is provided in Supplementary Table S2.

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**Table 3.** Top NMR Metabolites Associated With Macular Region Retinal Nerve Fiber Layer Thickness (mRNFL; $n$ = 10,584) and Macular Region Ganglion Cell Inner Plexiform Layer Thickness (mGCIPL; $n$ = 10,554) the UK Biobank.*

| Metabolites Associated With Thinner mRNFL Thickness (Microns) | | | | | | |
|---|---|---|---|---|---|---|
| | Model 1† | | Model 2† | | Model 3† | |
| Metabolite | Beta (95% CI) | NEF | Beta (95% CI) | NEF | Beta (95% CI) | NEF |
| Phospholipids in medium HDL | −0.04 (−0.11 to 0.04) | 0.99 | −0.17 (−0.25 to −0.09) | 6.3 E-04 | −0.15 (−0.24 to −0.07) | 0.011 |
| Total lipids in medium HDL | −0.02 (−0.10 to 0.05) | 0.99 | −0.16 (−0.24 to −0.08) | 2.1 E-03 | −0.15 (−0.24 to −0.07) | 0.013 |
| Cholesteryl esters in medium HDL | 0.00 (−0.08 to 0.07) | 0.99 | −0.15 (−0.23 to −0.07) | 0.011 | −0.15 (−0.24 to −0.07) | 0.017 |
| Cholesterol in medium HDL | 0.00 (−0.07 to 0.08) | 0.99 | −0.14 (−0.22 to −0.06) | 0.015 | −0.15 (−0.24 to −0.07) | 0.020 |
| Concentration of medium HDL particles | 0.00 (−0.07 to 0.07) | 0.99 | −0.15 (−0.22 to −0.07) | 0.012 | −0.15 (−0.23 to −0.06) | 0.027 |
| **Metabolites associated with thicker mGCIPL thickness (microns)** | | | | | | |
| | Model 1† | | Model 2† | | Model 3† | |
| Metabolite | Beta (95% CI) | NEF | Beta (95% CI) | NEF | Beta (95% CI) | NEF |
| Free cholesterol in small LDL | 0.28 (0.13 to 0.38) | 1.8 E-06 | 0.27 (0.17 to 0.37) | 6.2 E-06 | 0.19 (0.09 to 0.30) | 0.012 |
| Cholesteryl esters in medium VLDL | 0.30 (0.20 to 0.40) | 2.9 E-07 | 0.28 (0.18 to 0.39) | 1.5 E-06 | 0.20 (0.09 to 0.31) | 0.018 |
| Phospholipids in small LDL | 0.22 (0.12 to 0.32) | 6.2 E-04 | 0.24 (0.14 to 0.34) | 7.4 E-05 | 0.18 (0.07 to 0.28) | 0.031 |
| Free cholesterol in medium LDL | 0.24 (0.14 to 0.34) | 1.1 E-04 | 0.24 (0.14 to 0.34) | 8.6 E-05 | 0.17 (0.07 to 0.28) | 0.042 |
| Cholesterol in small LDL | 0.21 (0.11 to 0.31) | 1.3 E-03 | 0.23 (0.13 to 0.33) | 1.7 E-04 | 0.17 (0.07 to 0.28) | 0.044 |
| Concentration of large LDL particles | 0.24 (0.14 to 0.34) | 1.1 E-04 | 0.25 (0.15 to 0.25) | 4.9 E-05 | 0.18 (0.07 to 0.28) | 0.044 |

* The multiple linear regression models estimated differences in inner retina biomarker thickness with each 1 standard deviation increase in individual metabolites.

† **Model 1:** crude associations; **model 2:** adjusted for time since last meal/drink (≤4, 5–8, and 9+ hours), age, sex, race/ethnicity (White, Black, and other), season, and time of day of specimen acquisition; **model 3:** further adjustments for smoking status (never, past and current smoker), number of cigarettes smoked daily among current smokers, alcohol intake frequency (daily or almost daily, 3–4 times a week, 1–2 times a week, 1–3 times a month, special occasions only, and never), coffee and tea consumption (cups per day), physical activity (metabolic equivalent of task (MET) hours/week), Townsend deprivation index (range = −6 to 11; a higher index score indicates more relative poverty for a given residential area), body mass index (kg/m$^2$), systolic blood pressure (mm Hg), history of diabetes (yes or no), HbA1c (mmol/mol), history of cardiovascular disease, systemic beta-blocker use, use of statin drugs, use of oral steroids, spherical equivalents, and corneal compensated intraocular pressure (mm Hg).

We accounted for multiple comparisons by using the NEF statistic.

Abbreviations: CI, confidence interval; NEF, number of effective tests; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low density lipoprotein; NMR, nuclear magnetic resonance.

## Discussion

In this large study designed to evaluate a targeted set of metabolites simultaneously in relation to three vital ophthalmic biomarkers, we report many robust significant associations, but the effect sizes overall were small. These NMR spectroscopy results provide insights regarding how metabolites are related to IOP and inner retina layer biomarkers. First, whereas amino acids, specifically the BCAAs, were related to lower IOPccs, they were not related to inner retinal biomarker thicknesses. Second, in MSEA, the lipid components of lipoproteins (cholesterol, cholesteryl esters, triglycerides, and total lipids) were not associated with any of the three traits, whereas specific lipoproteins subclasses (especially HDL and LDL) were strongly associated with inner retinal biomarkers. HDL-related lipids were most consistently associated with thinner mRNFL, whereas LDL-related lipids were most consistently associated with thicker mGCIPL.

Although BCAAs were not related to inner retinal biomarker thicknesses values, they were associated with lower IOPccs. There is very little known about the role of BCAAs in aqueous humor dynamics. A prospective study performed among health professionals found that a higher dietary intake of BCAAs was not associated with POAG risk.[25] Plasma BCAAs were positively associated with exfoliation syndrome in a targeted mass spectroscopy metabolomics study of 16 cases and 18 controls.[26] This report is not consistent with our findings, as exfoliation syndrome is associated with high IOP. Thus, further studies are needed to confirm our results.

Our findings of increased albumin, increased lactate, and lower glutamine plasma levels being associated with higher IOP are consistent with a multi-ethnic Asian metabolomic study performed on an NMR spectroscopy platform.[4] Proteins gain access to the anterior chamber from the serum compartment via bulk diffusion through the anterior uveal tract.[27] Interestingly, IOP, but not central foveal thickness, decreases after hemodialysis, which reduces plasma protein load in chronic renal failure.[28] In addition, metabolic syndrome, which is characterized by glucose intolerance and dyslipidemia, has been associated with increased IOP.[29] Unlike Qian et al.,[4] we did not find a relationship between random plasma glucose and IOP.

We observed that lipids of various types had a positive association with increased IOPcc. This is consistent with the results from two UK cohorts showing a positive association among plasma total cholesterol, HDL-cholesterol, and LDL-cholesterol and higher IOP (measured using assays on the Beckman Coulter AU5800 platform).[5] The same study found an inverse relationship between plasma triglycerides and IOPcc in the UKBB (−0.05 mm Hg per 1 SD increase in triglyceride level; $P < 0.001$; $n$ = 94,323) but this finding was not replicated in our study where triglycerides were measured with NMR spectroscopy in a smaller sample (see Supplementary Table S1; 0.02 mm Hg per 1 SD increase; $P = 0.48$; $n$ = 28,195 participants; adjusted for covariates listed in model 3, Table 2). The relationship between

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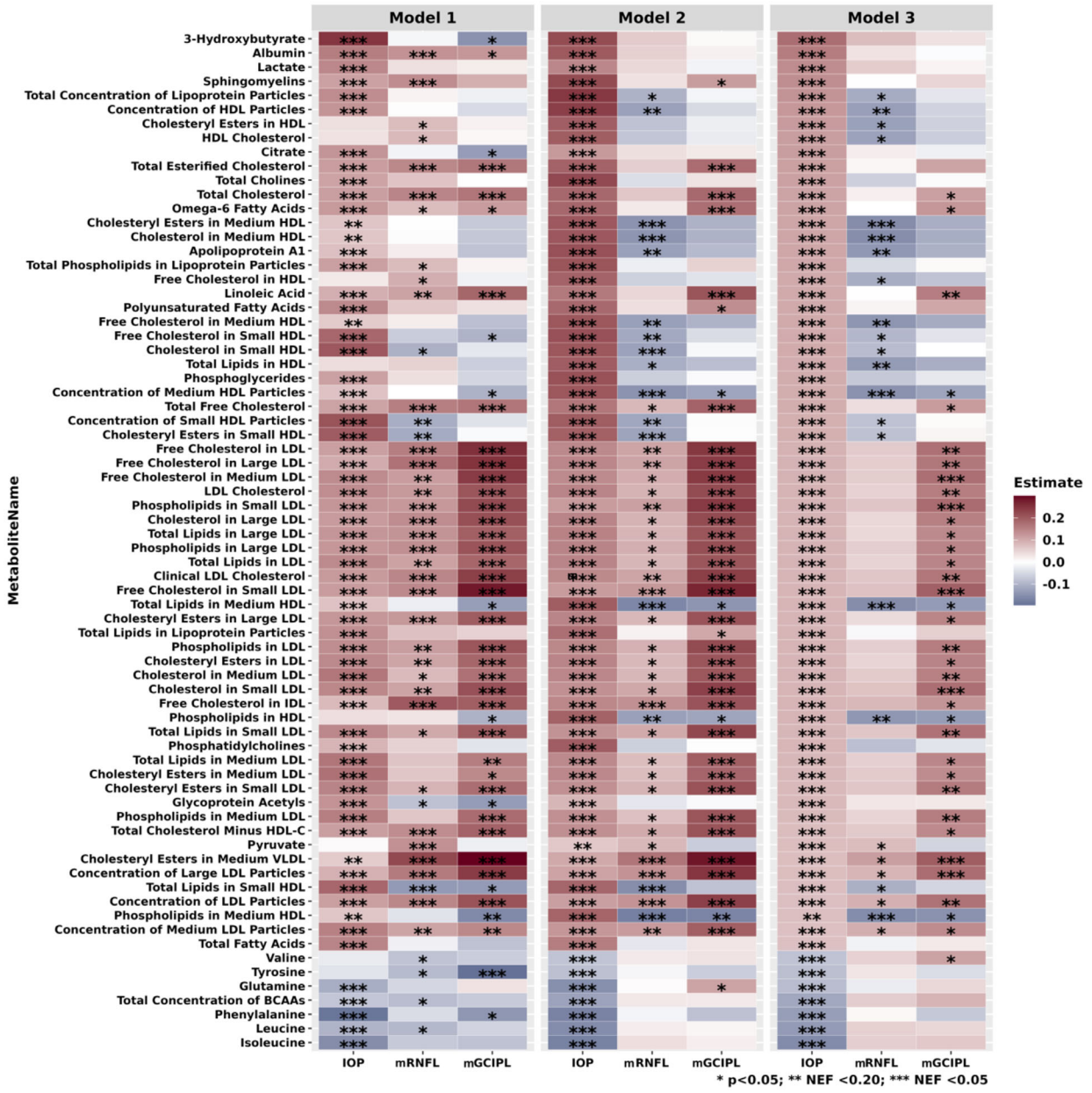


**Figure 2.** Heat map summarizing the relation between analytes and IOP (mm Hg, $n$ = 28,195), mRNFL thickness (µm, $n$ = 10,584), and mGCIPL thickness (µm, $n$ = 10,554) in the UK Biobank. IOP measures are corneal compensated. See footnotes of Tables 2 and 3 for a list of covariates used in the various linear multivariable models. We accounted for multiple comparisons by using the number of effective (NEF) test statistics. Actual effect sizes can be found in the tables and supplementary material. *Darker blue shades* refer to analytes with stronger inverse associations, while *darker red shades* refer to those with stronger positive associations. Only metabolites with NEF < 0.20 in model 3 are shown. Abbreviations: mm Hg – millimeters of mercury; µm – microns; IOP – intraocular pressure; mRNFL – macula-region retinal nerve fiber layer; mGCIPL – macula-region ganglion cell inner plexiform layer; LDL – low-density lipoprotein; HDL – high-density lipoprotein; IDL – intermediate-density lipoprotein; VLDL – very low-density lipoprotein; BCAA – branched-chain amino acids.

triglycerides and IOP is complex as triglycerides make up the core of many lipoproteins, and our MSEA indicated that some lipoproteins like medium HDL were nominally associated with higher IOP (NES = +1.4 mm Hg; FDR = 0.16; see Fig. 2 and Supplementary Table S2) and others like large VLDL was associated with lower IOP (NES = −2.2 mm Hg; FDR = 0.0025; see Fig. 2 and Supplementary Table S2).

Lipids are transported in the blood as lipoprotein particles that are classified by their density, size, and surface apoproteins. Lipoproteins contain a core of cholesteryl esters and mostly triglycerides enveloped in a phospholipid bilayer containing free cholesterol that is supported by surface apolipoproteins (apolipoprotein A1 for HDL and apolipoprotein B for LDL).[30] Lipoproteins have sizes that range from approximately 10 nm (HDL) to 1000 nm (chylomicrons). Interestingly, the retina can uptake LDL based on tracer studies performed in rats.[31] These findings may explain why several LDL-related lipids were associated with thicker mGCIPL, a finding consistent with

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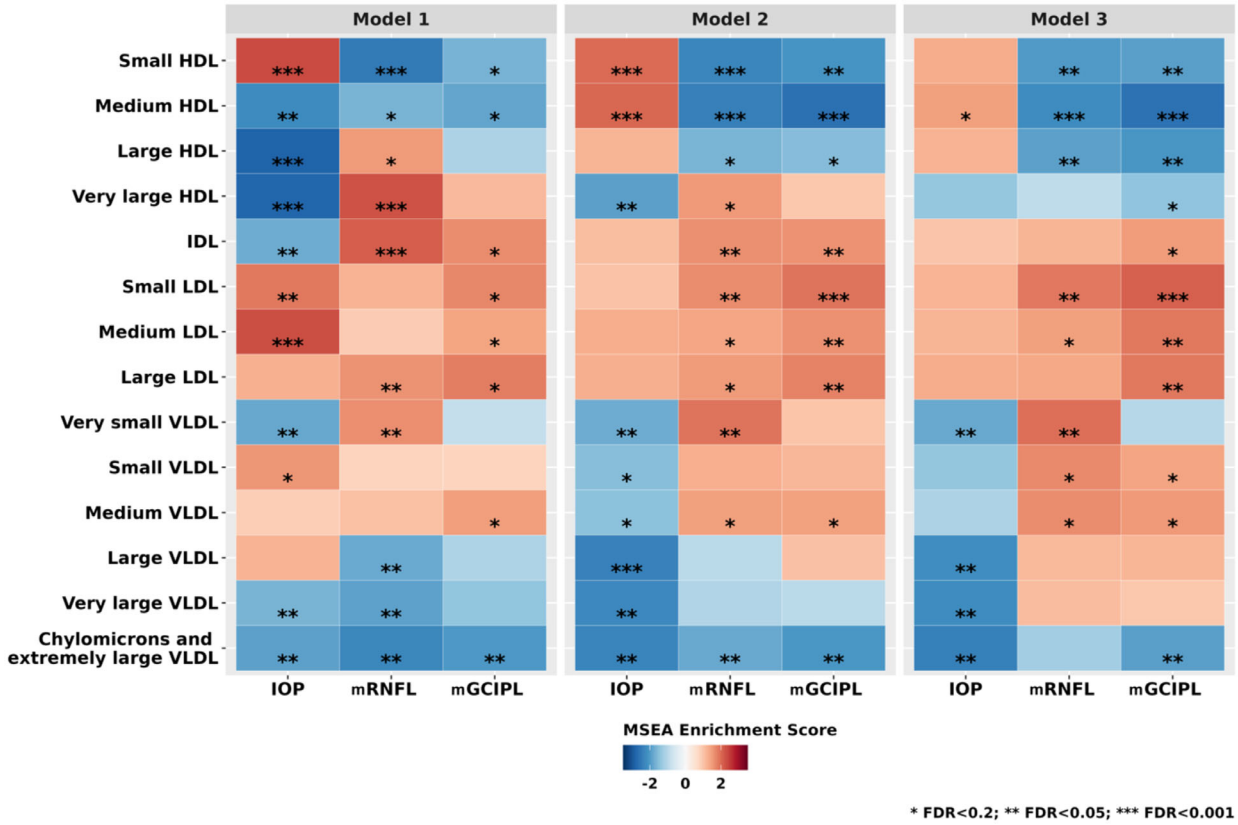


**Figure 3.** Heat map summarizing the relation between metabolite subclasses and IOP (mm Hg, $n = 28{,}195$), mRNFL thickness (µm, $n = 10{,}584$), and mGCIPL thickness (µm, $n = 10{,}554$) using metabolite set enrichment analysis in the UK Biobank. Metabolite set enrichment analysis (MSEA) results indicate the metabolite subclasses that are either more inversely (*blue*) or positively (*red*) associated with intraocular pressure (IOP), macular region retinal nerve fiber layer (mRNFL) thickness, or macular region ganglion cell inner plexiform layer (mGCIPL) thickness. IOP represents the corneal-compensated value. See footnotes of Tables 2 and 3 for a list of covariates used in multivariable linear models.

work performed by Rauscher et al.[6] On the other hand, apolipoprotein A1 and ABCA1 are prominently expressed in RGCs, particularly in the macula region,[32] where they may play a role in efficient HDL maturation and retinal efflux of cholesterol. Interestingly, ABCA1−/− mice have essentially no plasma HDL but total cholesterol accumulation in the retina leads to progressive RGC loss, suggesting that efficient HDL-mediated cholesterol retinal efflux is critical for RGC homeostasis.[33] Overall, the results of our study are consistent with the paradigm that LDL delivers lipids to the retina (producing slightly higher inner retinal thickness values), whereas HDL is responsible for retinal lipid efflux (producing slightly lower inner retinal thickness values). Nonetheless, should intracellular HDL levels decrease too much, as may occur in patients with POAG with the *ABCA1* rs2472493 [GG] genotype,[33,34] cholesterol buildup in the retina produces oxidative stress that causes pathologic RNFL thinning. Our work only explores plasma lipids, and more work is needed to understand how intracellular lipid processing influences retinal integrity.

These findings extend our published observations regarding the plasma metabolomic profile of POAG.[14] Plasma diglycerides and triglycerides were adversely related to POAG in the analysis of three US cohorts using a liquid chromatography-mass spectroscopy (LC-MS) platform. Those results were more robust for POAG with early paracentral visual loss. The LC-MS platform used precluded analysis of lipoproteins that carry diglycerides and triglycerides in the bloodstream as all proteins were precipitated from samples before metabolomic profiling. Our analysis here using NMR spectroscopy shows that plasma lipoproteins carrying DG and TG are indeed significant determinants of IOP and macula region inner retina structural parameters. One study suggests that plasma levels of small HDL is associated with reduced risk of POAG.[8] More study of NMR spectroscopy on pre-diagnostic plasma samples to further define the relationship between plasma lipoproteins and POAG stratified by visual field loss pattern is needed.

It is interesting to discuss our results in the context of statin use, which decreases plasma LDL, and their relationship to IOP and inner retinal biomarkers. A large study from the UK Biobank showed that current statin use was associated with borderline lower IOP (−0.6 mm Hg; $P = 0.10$) but with additional controls for total cholesterol and triglycerides, the association became borderline adverse (+0.05 mm Hg; $P = 0.17$).[35] In addition, statin users had thinner mRNFL in a multivariable model that included adjustment for plasma total cholesterol and triglyceride levels (−0.14 microns; $P = 0.03$).[35] Recent studies suggest that statins are not protective for patients with glaucoma,[35–38] perhaps

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because their effects on IOP are nominal. Furthermore, in our MESA analysis, various LDL subtypes were not associated with IOP, but they were associated with thicker mGCIPL and thicker mRNFL (see Figs. 2, 3, Supplementary Table S2). Although statins have unquestioned cardiovascular benefits,[39] the relation between statin use and glaucoma remains controversial.[40]

Strengths of this study include the use of plasma that was archived close to the time that ocular parameters were assessed, and the population was essentially free of glaucoma. IOP was measured on a single instrument type in a large number of participants. Studying the relationship between metabolites and OCT biomarkers is relatively novel. The sample sizes were quite large, allowing us to detect small effect sizes of statistical significance after correcting for multiple comparisons. We controlled for key covariates (e.g., body mass index [BMI], diabetes mellitus, and statin use), and our findings are consistent with prior data.

Several limitations of our data exist. Our study population was relatively homogeneous, with mostly European-derived White subjects; therefore, our findings may not be generalizable to other populations with different race and ethnicity compositions. IOP was measured only once with the ocular response analyzer (ORA) which yields less repeatable and more highly variable results compared to the Goldmann applanation tonometer (GAT).[41] Nonetheless, measuring IOP with GAT in such a large population would be challenging and such variability would likely drive results to the null. Additionally, there may have been residual confounding by other unmeasured factors, such as dietary patterns. A further potential limitation is that the blood samples were collected once and were taken at random times, although we do control for fasting status, season, and the time of day at sample collection. Furthermore, nearly two-thirds of people with metabolomic and IOP data did not have usable OCT data; nonetheless, the subjects in the IOP datasets and inner retinal biomarker datasets were comparable (see Table 1). In addition, this was a cross-sectional study whose results need confirmation in prospective studies. Finally, as stated above, we could not assess the functional impact of the metabolites on processes related to IOP level and inner retinal thickness values.

Overall, these results help to improve our understanding of various metabolites in relation to the outcomes of IOP, mRNFL, and mGCIPL. This NMR spectroscopic study highlighted the importance of various metabolites in relation to measures that are important in the glaucomatous process. More focus on how BCAAs might impact aqueous humor dynamics and how HDL/LDL impacts RGC integrity should be performed. Our data strongly implicate lipid metabolism in RGC homeostasis, and additional studies that further elucidate the mechanisms could lead to new targets for glaucoma prevention or therapies.

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