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Phospholipid Profiles of Control and Glaucomatous Human Aqueous Humor

Genea Edwards^a, Katyayini Aribindi^a, Yenifer Guerra^a, Richard K. Lee^a, and Sanjoy K. Bhattacharya^{a,*}

^aBascom Palmer Eye Institute, University of Miami School of Medicine, Miami, Florida, 33136

Abstract

To compare phospholipid (phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine and phosphatidylinositol) profiles of human control and glaucomatous aqueous humor (AQH).

AQH samples were procured during surgery from human POAG and control subjects (n=15 each). Samples were used following institutional review board approved protocols and adhering to the tenets of the Declaration of Helsinki. Lipid extraction was performed using a modification of the Bligh and Dyer method, protein concentrations were determined using the Bradford's method, and select samples were confirmed with Densitometry of PHAST gels. Lipids were identified and subjected to ratiometric quantification using a TSQ Quantum Access Max triple quadrupole mass spectrometer utilizing precursor ion scan (PIS) or neutral ion loss scan (NLS) using appropriate class specific lipid standards in a two step quantification process.

The comparative profiles of phosphatidylcholines, phosphatidylserines, phosphatidylethanolamines and phosphatidylinositols between control and glaucomatous AQH showed several species common between them. A number of unique lipids in all four phospholipid classes were also identified in control eyes that were absent in glaucomatous eyes and vice versa.

A number of phospholipids were found to be uniquely present in control, but absent in glaucomatous AQH and vice versa. Compared with a previous study of control and POAG red blood cells, a number of these phospholipids are absent locally (AQH).

Keywords

lipidomics; mass spectrometry; aqueous humor; glaucoma; phospholipids

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*Corresponding Author: McKnight Vision Research Building, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, 1638 NW 10th Avenue, Room 706A, Miami, Florida 33136, Tel: 305-482-4103, Fax: 305-326-6547, Sbhattacharya@med.miami.edu.

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1. Introduction

The glaucomas are a group of diseases that cause irreversible blindness frequently associated with an elevated intraocular pressure (IOP). Primary open angle glaucoma (POAG) is one of the most common forms. An estimated 60.5 million people worldwide suffer from glaucoma [1]. Elevation of IOP occurs due to pathologically increased resistance to aqueous humor drainage [2]. Lowering IOP is the only proven strategy for protecting the optic nerve from glaucomatous optic neuropathy. In addition to elevated IOP, diurnal fluctuation in IOP has also been found to be a risk factor for glaucoma development [3]. POAG is most frequently associated with increased elevated IOP [4] and more frequent fluctuation in IOP [3]. The aqueous humor is actively produced by ciliary epithelium [5, 6] and exits through the structures in the anterior chamber [4]. The elevated IOP is thought to be due to impeded outflow. The outflow is reduced in glaucoma due to increased resistance to outflow at the trabecular meshwork (TM), a filter like structure responsible for fluid flow regulation. The exact factor responsible for this increased resistance in the TM in glaucoma is poorly understood. Changes in the TM extracellular matrix (ECM) [7, 8] and the intrinsic elastic modulus of TM both at tissue and cell levels [9–11] have been demonstrated in glaucoma compared to physiologic conditions.

The main drainage pathway of aqueous humor (AQH) lies in the anterior chamber, which consists of the trabecular meshwork and the Schlemm's canal [12]. Two mechanisms of lowering IOP are decreasing the production of aqueous humor or increasing the outflow of aqueous humor. Aqueous outflow can be increased through two known pathways – the trabecular meshwork or the conventional, and the uveoscleral pathway. A number of pharmacological factors modulate aqueous outflow, for example, β -blockers and carbonic anhydrase inhibitors are known to decrease aqueous humor production. Another group of pharmacological factors, single classes of lipids known as prostaglandins were originally found to exist endogenously. These lipids were discovered in the iris and as a result were named irin [13, 14]. Although they were found to have IOP lowering ability by increasing aqueous humor outflow via the uveoscleral pathway, they also have significant side effects [13–18]. Further research into their mechanism of action provided insight into the existence of prostaglandin receptors in the uveoscleral pathway with greater concentration compared to that in the conventional pathway [19].

As noted above, the reason behind increased TM resistance in POAG remains poorly understood. The endogenous factors that regulate TM cell behavior and homeostasis also remain poorly understood. Importantly the presence of endogenous lipids in the AQH other than prostaglandins remains to be investigated. TM is constantly bathed in AQH and therefore it is plausible that factors in the AQH may play a modulatory role for TM cell behavior. Existence and the identification of different classes of endogenous lipids in the AQH largely remain to be investigated. A significant compositional change in the AQH may change the state of TM cell behavior and health of TM tissue. Until recently, suitable methods applicable to mixed lipids in very low amounts present in the AQH were critical barriers for their identification and simultaneous quantification. Recent developments in tandem mass spectrometry, bioinformatics and lipid databases have largely eliminated these barriers [20–24]. We present the results of profiling for four phospholipids classes namely:

phosphatidylcholines (PCs), phosphatidylserines (PSs), phosphatidylethanolamines (PEs) and phosphatidylinositols (PIs) in the aqueous humor and their comparative analyses between glaucomatous and control donors.

2. Materials and methods

2.1 Aqueous Humor Procurement

Control and POAG AQH were procured during glaucoma and cataract surgeries following institutional review board approved protocols and principles outlined in the Declaration of Helsinki were adhered to. A total of 15 control and 15 glaucomatous AQH samples (Supplemental Tables S1 and S2) were included for these studies. All AQH samples were immediately stored at -80°C until time of use. The mean age of donors was 69.8 ± 9.5 years and both genders were included for these studies.

2.2 Lipid Extraction

Aqueous humor samples were subjected to extraction of lipids using suitable and minimal modification of Bligh and Dyer method [25, 26]. The lower organic phase containing the extracted lipids was isolated and solvent dried with a Speed-Vac (Model 7810014; Labconco, Kansas City, MO). Samples were subsequently flushed with argon gas to prevent oxidation. Proteins recovered from the corresponding upper aqueous phase were quantified using Bradford's method [27]. A subset of protein samples were also subjected to densitometric quantification using bovine serum albumin (BSA) as a standard (amino acid quantified) after electrophoretic separation on a PHAST (GE Healthcare Bio-Sciences AB, Sweden) gel system [28]. We also repeated protein estimations using an amino acid analyzer after overnight digestion in hydrochloric acid following previously published protocols [29]. The protein amounts determined using amino acid analyzer was utilized in normalization of lipids per amount of proteins. In order to determine and ensure extraction efficiency, ovine wool cholesterol (molecular mass 386.7; catalog no. 700000; Avanti Polar Lipids, Alabaster, AL) [30] was premixed with AQH prior to extraction. All extractions and subsequent handling was made using glass vials to avoid contaminating impurities.

2.3 Mass Spectrometric Analysis

A triple quadrupole electrospray mass spectrometer (TSQ Quantum Access Max; Thermo Fisher Scientific, Pittsburgh, PA) was used for analysis of lipids in infusion mode using TSQ Tune software that is part of the Xcaliber 2.3 software package. Extracted lipids were dried and re-suspended in LC-MS grade acetonitrile: isopropanol (1:1). Samples were infused with a flow rate of $10\mu\text{l}/\text{min}$ and analyzed for 1.00 minute with a 0.500 second scan. Scans typically ranged from 200 m/z to 1000 m/z unless specified otherwise. A peak width was set at 0.7 and collision gas pressure was set at 1mTorr. Sheath gas (nitrogen) was set to 20 arbitrary units. Auxiliary gas (Argon) was set to 5 arbitrary units. For analyses of different phospholipid classes collision energy, spray voltage, and ion mode were set based on previous studies [20, 24, 26, 31]. Control and POAG AQH, $n=15$ each, were utilized for each of the four phospholipid classes analyzed. Class specific lipids were quantified using class specific quantitative lipid standards in two steps [20]. In the first step the most abundant lipids of the class were quantified using a class specific lipid standard and in the

second step, the quantification values determined using the first step were used for quantification of the identified low abundant lipid species [20, 24, 26]. The quantification lipid standards (all procured from Avanti Polar Lipids, Alabaster, AL) were the same as in our previous study on trabecular meshwork, namely 1,2-ditridecanoyl-sn-glycero-3-phosphocholine (molecular mass 649.89, catalog no. 850340) for PCs, 1,2-dioleoyl-sn-glycero-3-phospho-L-serine (molecular mass 810.03, catalog no. 840035) for PSs, 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (molecular mass 744.04, catalog no. 850725) for PEs and 1,2-dioleoyl-sn-glycero-3-phospho-(10-myoinositol) (molecular mass 880.15, catalog no. 850149) for PIs [26]. Quantification lipid standards are different than the lipid standard used for determination and normalization of extraction efficiency. The ovine wool cholesterol (molecular mass 386.7) was used for determination of extraction efficiency as stated above. About 10 scans each with and without internal standard (usually in the range of 0.1– 5 pmol) was performed for each sample. Ratiometric quantification was achieved using the MZmine 2.9 program. Lipid concentration was normalized to protein amount determined from the corresponding aqueous phase as described above.

2.4 Data Analysis

Representative spectra for each sample were carefully and manually inspected by two independent observers from 10 spectra collected for each sample with and without the internal standard (total 30 spectra) and then used for further analyses. Spectra was converted to netCDF files from Thermo RAW files using the Xcalibur 2.3 software suite, subsequently imported into MZmine 2.9 [32], and subjected to noise removal followed by analyses. A selected subset of data was also subjected to analysis in SimLipid version 3.1. The following steps were briefly used for quantification using MZmine 2.9. Thermo RAW files were imported into the MZmine program interface. Original RAW files were retained for future reference. Imported spectra were filtered by the Scan-by-Scan method. Masses were detected in centroid mode and noise levels were removed after the chromatogram was constructed following procedures as described previously [26]. Identification of lipids was achieved using a custom database created from the LipidMaps Database (LMSD). Salient features of phospholipid nomenclature have been presented (Supplemental Table S3). The identification so obtained indicates positions of double bonds and other features of lipids as present in the database and has been retained in our tables. However, our triple quadrupole data cannot verify all the detailed features. Thus, what we indicate in the table are the lipids as found in the database that have been matched by MZmine 2.9. Unique lipids (Table 1) are defined as a given lipid species found in only one group (control AQH or POAG AQH), and with a frequency of ≥ 2 donors. For common lipids species (Tables 2–5), the presence was recorded in both groups with at least a frequency of ≥ 2 donors in one group. Unique lipid experimental readings (the amount of lipid species) were found to be significantly different from 0.0 by one-sample t-test ($P < 0.05$). The common lipid species had statistically significant differences between control and POAG AQH by ANOVA. Scheffe's post hoc test demonstrated a statistically significant difference between control and POAG AQH samples for certain lipid species ($P < 0.05$).

3. Results

A representative phosphatidylcholine spectrum for aqueous humor samples from a control (Fig. 1A) and from a POAG donor (Fig. 1B) has been shown. Each of these spectra had a fixed amount (2 pmol) of a lipid standard added normalized to protein amount in the AQH, demonstrating the differences in AQH between the two donors. Total phospholipids in the AQH are reduced by 24.1% in POAG compared to control donor AQH (Table 6). The reduction in total PSs was higher (73.3%) while PCs underwent the least change (2.9%) in POAG compared to control donor AQH. Interestingly, total amount of PEs increased (79.4%) followed by PIs (30.0%) in POAG compared to control (Table 5). Comparison of lysophospholipid to phospholipid ratios between control and glaucomatous AQH (Supplemental Fig. 1) has been shown. The levels of lysophospholipids as a percent of total phospholipids were elevated in POAG AQH (Supplemental Fig. 1A) together with PS and PE in contrast to elevated levels of LysoPC and LysoPI in control AQH (Supplemental Fig. 1B–E).

3.1 Phosphatidylcholines of AQH

No unique PC species were found in either control or in POAG AQH (Table 1). The number of common PC species between control and glaucomatous AQH was 152 (Table 2).

3.2 Phosphatidylserines of AQH

No unique PS species were found in either control or in POAG AQH (Table 1). The number of common PS species in AQH was 141 (Table 3).

3.3 Phosphatidylethanolamines of AQH

There were 2 and 1 unique PE species identified in the control and glaucomatous (Table 1) AQH respectively while 142 species were common in both (Table 4). The most frequently observed unique PE species in control AQH were: PE-NMe₂(O-16:0/O-16:0) found in 2 donors and PE(18:1(9E)/18:1(9E)) found in 7 donors. The most frequently occurring PE species observed in glaucomatous AQH was: PE(15:0/20:3(8Z,11Z,14Z)) found in 4 donors (Supplemental Tables S1 and S2).

3.4 Phosphatidylinositols of AQH

One unique PI species was identified in the control AQH while two unique PI species were identified in the glaucomatous AQH (Table 1). There were 134 PI species in common between control and POAG AQH tissues (Table 5). The most frequently observed unique PI species in the control AQH were: PI(20:2(11Z,14Z)/22:4(7Z,10Z,13Z,16Z)) found in 6 donors. The most frequently occurring unique PI species in glaucomatous AQH were: PI(16:1(9Z)/22:4(7Z,10Z,13Z,16Z)) found in 6 donors and PI(O-18:0/0:0) found in 3 donors (Supplemental Tables S1 and S2).

4. Discussion

AQH outflow is impeded secondary to increased trabecular meshwork resistance in POAG [13], resulting in elevated IOP, which in turn, causes optic nerve damage. The factor that

increases resistance at the TM in POAG remains poorly understood. Whether factors exist in the AQH that bathes TM and affects TM cells behavior remains to be discovered. Initially characterized as smooth muscle contracting substrates, prostaglandin (PG) lipids were identified in the iris and these substances were collectively named irin in 1955 [13]. PG lipid analogs were subsequently found to be one of the most effective IOP reducing entities [33, 34]. However, the effects of other classes of lipids on IOP or outflow facility have just begun to be studied. A relationship between intraocular and systemic blood pressures is thought to exist and is potentially responsible for influencing the onset and/or the progression of POAG [35]. Vascular factors such as decreased optic nerve blood velocity and increased red blood cell aggregation have been thought to influence the progression of POAG by increasing intraocular pressure. These factors are influenced by lipids [36]. Findings such as significantly reduced levels of red blood cell and plasma eicosapentaenoic acid (EPA) as well as docosahexaenoic acid (DHA) compared with healthy patients may explain increased erythrocyte lipid membrane rigidity and aggregation observed in POAG patients. POAG patients had reduced phosphatidylcholine (PC) complexed DHA in erythrocytes [37]. Glaucoma patients are thought to have enhanced platelet aggregation, which impairs ocular blood flow. The EPA is a precursor of the vasodilator, anti-aggregatory and anti-inflammatory eicosanoids [36]. Regular intake of omega-3 fats is of fundamental importance to ocular health and supplementing the diet with EPA/DHA has been shown to reduce intraocular pressure in experimental animals [38, 39]. Also, lysophosphatidic acid (LPA) and sphingosine-1-phosphate (S1P) decrease aqueous humor outflow, indicating non – PG lipids are involved in regulation of AQH outflow as well [40]. As lysophospholipids have been implicated in aqueous outflow and in damage processes [41], we analyzed lysophospholipid to phospholipid ratios in AQH of control and POAG donors for total lipids (Supplementary Figure 1A) as well as for each class of phospholipids (Supplemental Fig. 1B–E). The lysophospholipid as a percent of total phospholipids indeed increases in glaucoma (Supplemental Fig. 1A) consistent with the possibility of their involvement in the damage process. This increase is mostly attributed to level of phosphatidylserines and phosphatidylethanolamines (Supplemental Fig. 1C and D). In contrast the lysoPC and lysoPI levels are higher in controls than in glaucoma (Supplemental Fig. 1B and E). The existence of members of all lipid classes and that of the lysophospholipids in the AQH remains to be investigated. The identification, quantification, and their changes in POAG will help investigate the roles select identified lipids play in TM cell biology.

Phospholipids are the main components of the membrane lipid bilayer that assist in creating cell boundaries as well as integrity of the cells for life processes. It is membrane phospholipids that create a hydrophobic environment for transmembrane protein function and communication. Some membrane lipids are part of lipid second messengers which are metabolized by enzymatic activity from phospholipid precursors [42]. However, there is increasing evidence that phospholipids may have a much larger role in the regulation of ocular mechanisms because of their presence in the aqueous humor and their subsequent changes under injurious circumstances. Studies have shown that growth factor-like glycerophosphate mediators of the LPA class are present in AQH as well as the lacrimal gland of a rabbit eye, and corneal injury has resulted in the increased production of these

mediators [43]. In the AQH itself, alkenyl-glycerophosphate has been identified in post injury AQH [43]. Lysophospholipase D (LysoPLD) activity of autotaxin was found significantly elevated in the aqueous humor of POAG patients in comparison with control aqueous humor, which suggests a high level of mechanistic importance in phospholipids in the aqueous humor [44]. Corneal wounds in rabbit eyes have shown a high enzyme activity of plasma lysoPLD, or autotaxin in both control and injured rabbit corneal tissue. However, levels of lysophosphatidylcholines were several times higher in injured corneal AQH samples in comparison with control AQH samples, suggesting that lysoPLD is released from corneal tissues into the AQH [45]. In addition, phosphatidylinositol (PIs) are one of the known classes of phospholipids undergoing significant changes during the administration of PG analogs for IOP lowering [46, 47]. Recent studies have also shown a significant difference in phospholipid species between human POAG and control trabecular meshwork tissue [48], yet the differences in phospholipid profiles in POAG AQH and control AQH have yet to be studied in further detail.

Endogenous lipids in the anterior chamber may be involved in regulation of aqueous outflow, outflow facility and IOP. The previous techniques such as various forms of chromatography and nuclear magnetic resonance necessitated (1) knowledge of several different chemistries due to different chemical behavior of different lipid species even within a class and, (2) requirement of large amounts (microgram quantities) of lipids for identification and characterization. Recent advances in mass spectrometry enable overcoming these two greatest hurdles towards high throughput determination of identities of phospholipids of all four classes (PC, PS, PE and PI) in the AQH. We have determined the endogenous phospholipids present in AQH using triple quadrupole mass spectrometry in precursor ion and neutral loss scan modes with parameters that have been well established in the lipid field [20]. Using these methods, we present two phospholipid species that have been determined as statistically significantly different; PC(16:0/26:2(5Z,9Z)) found in 4 control and 10 POAG donor AQH (Table 2) and PI(15:0/22:0) found in 8 control and 7 POAG donor AQH (Table 5). The same phosphatidylcholine and phosphatidylinositol species were found in the TM of control and POAG donor tissues [48]. Control and POAG TM tissues have little difference in the amount of PC(16:0/26:2(5Z,9Z)); however, control and POAG AQH have a significant difference, with the control AQH having a much higher amount than POAG. PI(15:0/22:0) was found in a much higher amount in control AQH, than in POAG AQH, but in much lower amounts in control TM than in POAG TM. Data suggests these phosphatidylcholine and phosphatidylinositol species undergoes a switch in average amounts in correlation with the development of POAG, which alludes to PC(16:0/26:2(5Z,9Z)) and PI(15:0/22:0) moving into the AQH from the TM under normal physiological conditions, but their inhibited movement with development of POAG. These results allude to a possibility of an unnatural buildup of PC(16:0/26:2(5Z,9Z)) and PI(15:0/22:0) in POAG TM, and thus, much lower amounts in the AQH. Further investigation of whether PI(15:0/22:0) and PC(16:0/26:2(5Z,9Z)) are limited to the TM-AQH system and how exactly these phospholipids come to appear in two different tissues remains pure speculation at the present moment but are important questions for future experimentation.

In the current investigation, all AQH samples were collected locally, frozen immediately and were stored at -80°C until used for analyses. AQH samples were subjected to only one thawing cycle prior to their use. Our control analyses with cornea and other anterior chamber tissue/fluids from mammalian model systems (porcine, bovine and a select subset of humans) showed a decrease of phospholipid species but no selective absence or appearance of a phospholipid species as a function of storage up to a week at 4°C in PBS or Optisol GS [49] and no alteration when immediately stored in -80°C and thawed only once (Bhattacharya, SK and Aribindi, K.; unpublished observations), our observations parallels that for mass spectrometric analyses of serum [50], where repeated freeze-thaw cycles have been previously shown to affect small molecules and peptides. AQH is likely to provide an accurate depiction into the differential lipid profile between control and diseased samples because of the local control of sample from collection to analyses. Three other factors are intrinsically likely to broadly affect the lipid profiles apart from biochemical individuality of the donors: (a) confounding factors of diseases or disorders that are well known to affect lipid profiles such as hyperlipidemia, (b) use of drugs by the donors such as statins and (c) confounding factors of diseases that are per se not lipid related such as diabetes. Previously, use of statins have found not to affect the phospholipid profiles in the lens and conjectured not to affect that in the ocular tissues [51]. Careful analyses of statin users reflect very little changes in phospholipids systemically [52] supporting that phospholipids are probably not significantly affected in statin users. Nevertheless, information as to whether or not a donor was on statins has been provided for the phospholipid species that were classified as unique identifications.

The triple quadrupole instrument used here has a resolution of 1 atomic mass unit (at 0.7FWHM), which is a first pass attempt to profile all phospholipid species. Further high-resolution mass spectrometry will enable these identifications with greater conformity. In the future, the unique species identified here will need to be carefully characterized using different collision energies and high-resolution mass spectrometry. A comprehensive comparison between TM and AQH, along with other tissues including blood and optic nerve will provide insight about systematic and local changes occurring in glaucoma as well as complications from confounding factors. A possibility of an applied outcome from this research also looms in the horizon. Continued research in PG analogs beyond their original discovery in the iris [13] led to their development as effective IOP reducing topical medications like latanoprost and travoprost [53]. Lowering IOP by increasing the outflow via the conventional pathway will be close to normal physiology [54, 55]. Apart from pilocarpine, a muscarinic agonist of inferior efficacy compared to prostaglandins with significant side-effects, no other drugs are available to enhance aqueous outflow via the TM pathway. The development of additional IOP lowering drugs, especially those through which aqueous drainage is increased via the conventional outflow pathway, is needed. Endogenous lipids other than prostaglandins present in the AQH might have some potential in this direction and only future continued research will reveal whether this is a viable possibility.

In conclusion, future work will reveal biological consequences of local alteration of phospholipids in the AQH as well as the TM region and insight into their contribution in pathology. Profiling of lipids is also expected to help expand databases (perhaps

infinitesimally incremental) enabling the synthesis of interesting lipid species and their screening to determine the biological roles that they might play in the anterior segment of the eye.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Phospholipid profile comparison of human control and glaucomatous aqueous humor.
- Mass spectrometric showed several phospholipid species common between them.
- A number of unique lipid species in four phospholipid classes were also identified.
- Some were uniquely present in control, but absent in glaucomatous and vice versa.

Figure 1.

Representative electrospray ionization mass spectrometric analysis of the phosphatidylcholine (PC) class of lipids in human aqueous humor (AQH) analyzed in positive-ion mode. (A) Representative spectra of human control and (B) glaucomatous AQH as indicated with internal standard (arrowhead; m/z ratio of 650.6) that has been used for ratiometric quantification. Precursor ion scan for m/z 600–900 has been shown. Arrow shows presence of a unique species in control AQH.

Table 1

Unique Phospholipid Species in Control and Glaucomatous Aqueous Humor

	m/z*	Average lipid amount (pmol per species/μg protein)	Donor frequency	LIPIDMAPS ID**	PUBCHEM ID
Phosphatidylethanolamines					
Control Aqueous Humor					
PE-NMe2(O-16:0/O-16:0)	692.55	0.002	2	LMGP02040007	14714332
PE(18:1(9E)/18:1(9E))	743.46	25.085	7	LMGP02010039	14714000
Glaucomatous Aqueous Humor					
PE(15:0/20:3(8Z,11Z,14Z))	727.53	26.092	4	LMGP02010466	123061712
Phosphatidylinositols					
Control Aqueous Humor					
PI(20:2(11Z,14Z)/22:4(7Z,10Z,13Z,16Z))	938.93	0.119	6	LMGP06010555	123065574
Glaucomatous Aqueous Humor					
PI(16:1(9Z)/22:4(7Z,10Z,13Z,16Z))	884.56	0.008	6	LMGP06010193	123065212
PI(O-18:0/0:0)	587.67	0.003	3	LMGP06060002	123066196

* A representative mass/charge ratio is presented (variations in m/z was reconciled by MZmine 2.9).

** The lipid species identification is based on Lipidmaps database, used as a *.csv file for bioinformatic analyses with MZmine 2.9 program. Average standard non-normalized dataset is presented here.

Table 2

Common lipid species between control and glaucomatous aqueous humor

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PC(10:0/10:0)	565.23	0.028	0.057	5	0.195	0.423	5
PC(10:0/16:0)	649.76	0.894	2.120	7	6.719	11.638	3
PC(10:0/18:0)	677.90	0.010	0.020	7	0.437	1.071	6
PC(10:0/18:1(9Z))	675.21	0.012	0.009	5	0.005	0.009	5
PC(10:0/18:2(9Z,12Z))	673.30	0.008	0.015	10	0.004	0.005	6
PC(10:0/19:0)	691.26	0.006	0.007	9	0.640	1.152	4
PC(10:0/20:0)	705.56	0.153	0.331	6	0.237	0.551	7
PC(10:0/21:0)	719.74	0.002	0.004	7	0.004	0.005	2
PC(10:0/22:0)	733.62	0.008	0.009	8	0.270	0.743	8
PC(10:0/23:0)	747.19	0.496	1.359	8	5.536	14.646	7
PC(10:0/24:0)	761.33	0.008	0.011	5	0.294	0.506	3
PC(10:0/25:0)	775.04	17.117	34.117	4	0.458	0.990	8
PC(10:0/4:0)	481.11	0.261	0.612	6	1.311	2.931	5
PC(11:0/11:0)	593.60	0.004	0.008	7	0.180	0.538	9
PC(12:0/12:0)	621.48	0.003	0.006	4	14.901	36.165	6
PC(12:0/13:0)	636.43	0.031	0.008	4	0.911	1.711	4
PC(12:0/14:1(9Z))	647.62	0.006	0.012	6	0.502	0.870	3
PC(12:0/15:1(9Z))	661.40	0.001	0.001	4	0.228	0.393	3
PC(12:0/17:2(9Z,12Z))	687.15	0.013	0.028	8	0.038	0.082	5
PC(12:0/18:1(9Z))	703.10	1.702	4.492	7	0.140	0.314	5
PC(12:0/18:2(9Z,12Z))	701.21	0.037	0.078	6	0.172	0.378	5
PC(12:0/18:3(6Z,9Z,12Z))	699.04	3.420	4.836	2	0.028	0.063	6
PC(12:0/18:4(6Z,9Z,12Z,15Z))	697.29	0.001	0.001	5	0.097	0.273	8
PC(12:0/20:5(5Z,8Z,11Z,14Z,17Z))	723.33	0.006	0.007	4	0.060	0.135	5
PC(12:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	750.11	0.002	0.002	2	0.213	0.522	6
PC(12:0/26:0)	817.45	0.019	0.032	7	0.875	1.957	5

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PC(13:0/13:0)	651.22	0.001	0.002	2	0.009	0.007	6
PC(13:0/18:2(9Z,12Z))	714.60	0.004	0.005	4	0.353	0.789	5
PC(13:0/18:3(6Z,9Z,12Z))	713.31	0.004	0.006	2	2.039	4.078	4
PC(13:0/18:4(6Z,9Z,12Z,15Z))	711.33	11.139	27.271	6	0.745	1.491	4
PC(13:0/20:3(8Z,11Z,14Z))	741.47	0.007	0.012	5	0.053	0.127	7
PC(13:0/20:4(5Z,8Z,11Z,14Z))	739.49	0.798	1.781	5	0.049	0.122	7
PC(13:0/20:5(5Z,8Z,11Z,14Z,17Z))	737.11	2.261	3.915	3	13.326	15.468	2
PC(13:0/22:2(13Z,16Z))	771.14	0.428	1.182	8	0.037	0.064	3
PC(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	763.60	0.002	0.003	9	0.307	0.643	6
PC(14:0/18:1(11Z))	731.37	0.004	0.005	7	0.048	0.120	7
PC(14:0/18:2(11Z,14Z))	729.05	0.004	0.007	7	0.016	0.031	4
PC(14:0/18:3(9Z,12Z,15Z))	727.56	0.003	0.006	7	0.731	1.631	5
PC(14:0/18:4(6Z,9Z,12Z,15Z))	725.53	0.002	0.002	3	15.431	48.654	10
PC(14:0/2:0)	508.81	0.713	1.838	7	0.240	0.480	4
PC(14:0/20:4(5Z,8Z,11Z,14Z))	753.66	0.021	0.025	8	13.381	23.176	3
PC(14:0/20:5(5Z,8Z,11Z,14Z,17Z))	751.60	0.001	0.002	6	1.32E-04	1.85E-04	4
PC(14:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	777.99	0.011	0.021	7	0.054	0.092	5
PC(14:0/24:1(15Z))	815.21	0.005	0.007	5	18.690	45.082	6
PC(14:0/26:0)	845.82	0.007	0.008	7	0.934	2.088	5
PC(14:1(9Z)/0:0)	464.88	0.209	0.491	6	7.80E-05	7.80E-05	3
PC(15:0/18:1(11Z))	745.25	0.006	0.011	5	0.124	0.210	6
PC(15:0/18:2(9Z,12Z))	743.67	0.006	0.011	5	0.316	0.699	5
PC(15:0/20:3(8Z,11Z,14Z))	769.68	0.001	0.001	4	0.001	0.002	7
PC(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	765.03	3.06E-05	4.33E-05	2	0.005	0.005	3
PC(15:0/22:1(11Z))	801.66	0.731	1.265	3	0.106	0.181	5
PC(15:0/22:2(13Z,16Z))	799.05	0.002	0.003	5	0.002	0.003	4
PC(15:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	791.70	0.002	0.002	5	0.281	0.741	7
PC(15:1(9Z)/0:0)	478.55	0.038	0.074	5	0.295	0.719	6
PC(15:1(9Z)/22:2(13Z,16Z))	797.36	0.001	0.001	3	0.001	0.002	4

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PC(15:1(9Z)/22:4(7Z,10Z,13Z,16Z))	793.68	0.014	0.032	7	2.52E-04	2.67E-04	2
PC(16:0/15:1(14))	717.21	0.008	0.010	7	0.351	0.651	8
PC(16:0/18:1(9Z))	760.03	0.131	0.339	7	0.327	0.771	6
PC(16:0/18:2(10E,12Z))	757.25	3.944	10.347	7	0.099	0.140	5
PC(16:0/18:3(6Z,9Z,12Z))	755.40	0.005	0.011	5	0.001	0.002	5
PC(16:0/2:0)	537.31	31.766	84.034	7	0.291	0.539	8
PC(16:0/20:3(5Z,8Z,11Z))	784.42	0.023	0.033	2	0.324	0.725	5
PC(16:0/20:4(5Z,8Z,11Z,14Z))	781.94	0.003	0.005	10	0.789	1.762	5
PC(16:0/20:5(5Z,8Z,11Z,14Z,17Z))	779.30	0.005	0.011	5	1.47E-04	8.69E-05	5
PC(16:0/22:4(7Z,10Z,13Z,16Z))	809.88	0.011	0.012	6	16.791	34.734	5
PC(16:0/22:5(4Z,7Z,10Z,13Z,16Z))	807.09	0.012	0.024	7	0.003	0.004	6
PC(16:0/22:6(4E,7E,10E,13E,16E,19E))	805.07	0.001	0.002	4	0.065	0.118	6
PC(16:0/23:5(8E,11E,14E,17E,20E))	821.36	0.001	0.002	5	15.670	37.818	6
PC(16:0/24:1(15Z))	843.44	0.003	0.005	4	0.385	0.483	5
PC(16:0/26:0)	873.72	0.002	0.003	6	0.457	0.725	4
PC(16:0/26:2(5Z,9Z))	869.19	1.581	3.107	4	0.269	0.568	10
PC(16:0/3:0)	551.33	0.006	0.010	6	0.004	0.008	5
PC(16:0/3:1(2E))	548.89	0.394	1.168	9	0.079	0.171	5
PC(16:0/5:0(COOH))	609.52	0.004	0.009	8	0.148	0.441	9
PC(16:0/5:0)	578.94	21.179	56.017	7	0.006	0.008	2
PC(16:0/5:1(4E))	577.42	0.001	0.002	4	0.001	0.002	7
PC(16:0/9:0(COOH))	665.49	0.004	0.008	6	0.470	0.938	4
PC(16:1(9E)/0:0)	492.97	0.351	0.681	4	0.020	0.033	3
PC(16:1(9Z)/2:0)	534.97	0.001	0.002	4	4.34E-05	5.07E-05	3
PC(16:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	803.05	0.012	0.013	2	0.352	0.861	6
PC(17:0/10:0)	663.62	0.013	0.023	6	0.247	0.650	9
PC(17:0/18:1(9Z))	773.00	0.003	0.005	8	0.303	0.797	7
PC(17:0/20:4(5Z,8Z,11Z,14Z))	796.00	0.004	0.009	6	0.407	0.875	6
PC(17:0/22:2(13Z,16Z))	827.52	0.660	1.603	6	1.076	2.058	4

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PC(17:0/22:4(7Z,10Z,13Z,16Z))	823.75	0.006	0.013	4	9.19E-05	4.94E-05	3
PC(17:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	819.66	0.004	0.008	9	0.001	0.002	4
PC(17:1(10Z)/0:0)	506.81	0.002	0.002	6	0.683	1.183	3
PC(17:1(9Z)/22:2(13Z,16Z))	825.91	0.008	0.011	2	0.001	0.002	5
PC(17:2(9Z,12Z)/0:0)	505.63	0.004	0.003	4	0.653	1.200	4
PC(18:0/18:0)	789.35	0.005	0.007	9	0.251	0.466	8
PC(18:0/18:1(11Z))	787.41	0.001	0.001	4	0.327	0.794	6
PC(18:0/18:2(10Z,12Z))	785.87	0.008	0.017	6	0.001	0.002	4
PC(18:0/20:2(11Z,14Z))	813.26	0.004	0.005	2	0.113	0.291	7
PC(18:0/20:3(5Z,11Z,14Z))	811.65	0.002	0.004	6	0.006	0.009	2
PC(18:0/22:3(10Z,13Z,16Z))	840.05	0.006	0.013	8	0.398	0.787	5
PC(18:0/22:4(7Z,10Z,13Z,16Z))	837.65	4.358	8.714	4	0.257	0.444	3
PC(18:0/22:5(4Z,7Z,10Z,13Z,16Z))	835.24	0.004	0.006	6	0.347	0.910	7
PC(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	833.39	0.004	0.005	6	0.097	0.232	6
PC(18:0/24:1(15Z))	871.69	0.002	0.004	3	0.003	0.005	5
PC(18:1(11Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	831.52	0.006	0.010	8	0.326	0.722	5
PC(18:1(9E)/2:0)	562.92	7.48E-07	9.94E-07	4	0.083	0.227	10
PC(18:1(9Z)/4:0)	591.03	0.059	0.129	6	0.067	0.131	4
PC(18:2(2E,4E)/0:0)	518.98	0.001	0.003	8	0.002	0.004	4
PC(18:2(9Z,12E)/17:2(9Z,11E))	767.74	22.423	54.916	6	0.548	0.940	3
PC(18:2(9Z,12Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z)829.35		0.004	0.007	8	0.419	1.020	6
PC(18:3(9Z,12Z,15Z)/0:0)	516.98	0.006	0.008	7	0.006	0.014	6
PC(18:4(9E,11E,13E,15E)/0:0)	514.66	2.576	5.735	5	0.080	0.121	6
PC(19:0/22:1(11Z))	856.88	0.006	0.006	6	0.067	0.133	4
PC(19:0/22:2(13Z,16Z))	855.87	2.40E-04	3.83E-04	3	0.001	0.002	7
PC(19:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	847.82	0.003	0.007	9	0.205	0.446	7
PC(19:1(9Z)/22:4(7Z,10Z,13Z,16Z))	848.70	0.004	0.006	2	6.92E-05	5.39E-05	5
PC(19:3(10Z,13Z,16Z)/0:0)	531.83	4.098	8.468	9	0.602	1.041	8
PC(20:0/20:2(11Z,14Z))	841.27	0.001	0.001	4	0.261	0.522	4

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PC(20:0/22:4(7Z,10Z,13Z,16Z))	865.66	0.001	0.001	2	0.044	0.109	7
PC(20:0/22:5(7Z,10Z,13Z,16Z,19Z))	863.72	0.230	0.667	9	0.220	0.532	7
PC(20:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	861.72	0.004	0.004	5	0.713	0.980	5
PC(20:0/24:1(15Z))	899.06	0.014	0.009	2	0.594	1.018	3
PC(20:1(11Z)/22:2(13Z,16Z))	867.67	2.63E-05	3.60E-05	3	1.16E-05	1.32E-05	4
PC(20:4(5Z,8Z,11Z,14Z)/0:0)	543.05	0.010	0.011	5	0.297	0.782	7
PC(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	540.97	5.361	10.714	4	2.534	6.704	7
PC(20:5(5Z,8Z,11Z,14Z,17Z)/22:5(7Z,10Z,13Z,16Z,19Z))	882.82	0.001	0.002	7	1.482	2.386	7
PC(20:5(5Z,8Z,11Z,14Z,17Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	882.82	3.966	11.205	8	0.620	1.000	6
PC(21:0/22:1(11Z))	885.21	0.003	0.004	6	0.476	1.064	5
PC(21:0/22:2(13Z,16Z))	882.82	0.008	0.012	6	0.742	1.285	3
PC(21:0/22:4(7Z,10Z,13Z,16Z))	879.68	0.003	0.003	6	0.201	0.575	10
PC(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	876.11	0.006	0.007	8	0.506	1.132	5
PC(22:0/22:4(7Z,10Z,13Z,16Z))	893.46	0.018	0.029	5	0.382	1.074	9
PC(22:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	890.06	0.002	0.002	6	0.598	1.333	5
PC(22:1(11Z)/22:2(13Z,16Z))	896.25	0.003	0.005	4	0.003	0.005	6
PC(22:1(11Z)/22:4(7Z,10Z,13Z,16Z))	891.42	0.001	0.001	5	0.001	0.001	5
PC(22:1(13E)/22:1(13E))	896.91	0.017	0.015	2	1.396	2.196	3
PC(22:2(13Z,16Z)/0:0)	574.78	0.008	0.007	5	0.206	0.435	5
PC(22:4(7Z,10Z,13Z,16Z)/0:0)	571.33	35.071	60.745	3	0.373	0.697	8
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	567.53	1.431	3.196	5	0.909	1.575	3
PC(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	878.13	0.012	0.013	3	0.006	0.015	7
PC(23:0/18:0)	859.57	0.003	0.004	6	0.003	0.006	3
PC(24:0/0:0)	607.61	0.007	0.014	7	0.131	0.244	6
PC(25:0/18:0)	887.91	0.008	0.007	4	0.716	1.709	6
PC(5:0/5:0)	425.12	0.003	0.003	6	0.194	0.397	8
PC(6:0/6:0)	453.41	0.007	0.013	4	0.046	0.096	5
PC(6:2(2E,4E)/6:2(2E,4E))	445.27	0.004	0.004	5	0.610	1.608	7
PC(6:2(3E,5E)/14:2(11E,13E))	556.97	17.064	48.247	8	11.401	33.557	9

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PC(8:2(2E,4E)/8:2(2E,4E))	501.42	0.006	0.006	7	0.125	0.333	9
PC(O-10:1(9E)/2:0)	436.97	0.007	0.012	4	0.167	0.400	7
PC(O-11:1(10E)/2:0)	450.87	0.812	1.785	5	0.559	1.408	8
PC(O-12:0/2:0)	467.14	0.898	2.165	6	0.799	1.760	5
PC(O-12:0(O-1:0)	440.00	0.006	0.007	5	1.673	2.729	6
PC(O-14:0/2:0)	495.41	0.005	0.009	4	14.599	29.026	4
PC(O-16:0/2:0)	522.86	1.951	3.109	6	5.254	13.897	7
PC(O-16:1(11Z)/2:0)	520.87	0.001	0.001	3	0.169	0.279	5
PC(O-18:2(9Z,12Z)/2:0)	547.08	0.010	0.007	3	0.0002	0.0001	3
PC(O-8:0/2:0)	411.34	0.024	0.059	9	0.100	0.133	6

*The lipid species identification is based on Lipidmaps database, used as a *.csv file for bioinformatic analyses with MZmine 2.9 program.

**A representative mass/charge ratio is presented (variations in m/z was reconciled by MZmine 2.9). Average standard non-normalized dataset is presented here. For some lipid species identified, standard error of mean could not be calculated due to lack of presence in all samples.

Table 3

Common lipid species between control and glaucomatous aqueous humor

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PS(10:0/10:0)	567.38	0.878	2.080	10	5.847	12.149	9
PS(12:0/0:0)	440.94	14.800	43.733	10	5.666	9.098	10
PS(12:0/12:0)	623.47	0.714	1.520	9	6.280	11.708	9
PS(12:0/13:0)	637.56	0.746	1.668	11	5.254	8.527	10
PS(12:0/14:1(9Z))	649.53	0.868	1.888	9	2.276	5.312	7
PS(12:0/15:0)	665.73	1.692	3.625	8	21.045	53.991	8
PS(12:0/15:1(9Z))	662.73	6.750	19.875	10	1.388	4.048	10
PS(12:0/16:1(9Z))	676.97	85.945	253.572	9	53.925	154.644	9
PS(12:0/17:0)	693.70	2.222	4.995	13	4.394	9.066	8
PS(12:0/17:1(9Z))	691.21	34.288	90.698	7	4.239	8.825	10
PS(12:0/17:2(9Z,12Z))	688.80	4.995	13.589	9	2.310	5.790	11
PS(12:0/18:2(9Z,12Z))	703.24	13.098	35.410	8	6.101	11.455	8
PS(12:0/18:3(6Z,9Z,12Z))	700.64	2.082	3.448	5	2.043	4.324	9
PS(12:0/18:4(6Z,9Z,12Z,15Z))	698.76	0.512	0.901	10	3.350	8.332	10
PS(12:0/19:0)	722.43	10.936	29.820	8	5.651	9.659	7
PS(12:0/20:1(11Z))	733.17	0.709	1.566	9	2.976	7.787	11
PS(12:0/20:2(11Z,14Z))	731.30	28.350	83.670	9	2.088	3.654	7
PS(12:0/20:3(8Z,11Z,14Z))	729.00	13.816	42.656	10	2.973	8.838	10
PS(12:0/20:4(5Z,8Z,11Z,14Z))	727.08	1.267	2.733	8	2.021	5.281	11
PS(12:0/20:5(5Z,8Z,11Z,14Z,17Z))	725.21	0.502	0.915	6	1.817	3.996	12
PS(12:0/21:0)	749.85	24.075	75.376	10	4.193	12.125	10
PS(12:0/22:2(13Z,16Z))	758.82	15.156	43.647	9	1.405	2.821	8
PS(12:0/22:4(7Z,10Z,13Z,16Z))	755.54	1.044	2.545	10	5.027	8.563	6
PS(13:0/17:1(9Z))	705.99	0.598	1.056	8	2.509	4.681	8
PS(13:0/18:2(9Z,12Z))	717.22	7.906	19.357	10	2.080	5.079	9
PS(13:0/18:3(6Z,9Z,12Z))	715.14	0.041	0.072	9	2.438	5.357	12

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PS(13:0/18:4(6Z,9Z,12Z,15Z))	713.15	0.170	0.322	10	3.307	5.414	7
PS(13:0/20:1(11Z))	747.07	0.860	1.964	9	3.103	5.171	7
PS(13:0/20:2(11Z,14Z))	745.24	0.668	1.098	5	1.270	3.660	11
PS(13:0/20:3(8Z,11Z,14Z))	742.91	1.566	2.933	7	2.885	5.301	9
PS(13:0/20:4(5Z,8Z,11Z,14Z))	740.74	20.777	54.213	11	2.353	4.144	8
PS(13:0/20:5(5Z,8Z,11Z,14Z,17Z))	738.77	0.668	1.550	10	0.885	2.460	11
PS(13:0/22:0)	777.11	17.347	49.697	9	15.830	34.760	7
PS(13:0/22:1(11Z))	775.30	0.850	1.823	8	4.007	8.601	8
PS(13:0/22:2(13Z,16Z))	773.21	20.777	50.325	7	3.671	11.110	12
PS(13:0/22:4(7Z,10Z,13Z,16Z))	769.67	1.161	2.479	8	7.320	17.388	11
PS(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	765.43	1.410	3.216	9	1.750	4.225	14
PS(14:0/0:0)	468.82	0.457	0.811	8	6.397	17.688	9
PS(14:0/12:0)	652.10	0.421	0.885	8	3.463	9.141	12
PS(14:0/14:0)	679.48	1.153	2.283	7	3.961	7.735	9
PS(14:0/20:3(8Z,11Z,14Z))	757.78	0.005	0.007	4	0.015	0.014	6
PS(14:1(9Z)/0:0)	466.90	0.736	1.483	8	2.549	5.585	12
PS(14:1(9Z)/14:1(9Z))	674.83	0.367	0.847	10	7.504	13.730	7
PS(14:1(9Z)/20:4(5Z,8Z,11Z,14Z))	753.40	23.352	64.524	8	2.149	4.488	11
PS(14:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	751.27	0.168	0.274	3	0.021	0.035	4
PS(14:1(9Z)/22:2(13Z,16Z))	785.42	1760.080	3935.379	5	1.333	2.303	5
PS(14:1(9Z)/22:4(7Z,10Z,13Z,16Z))	780.78	0.140	0.276	12	0.311	0.619	9
PS(15:0/0:0)	483.48	0.021	0.053	8	5.440	13.711	10
PS(15:0/20:3(8Z,11Z,14Z))	771.59	0.005	0.007	4	0.024	0.018	2
PS(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	767.60	369.017	1043.694	8	0.034	0.043	4
PS(15:0/22:1(11Z))	802.96	0.542	1.304	12	1.830	5.086	8
PS(15:0/22:2(13Z,16Z))	800.83	0.405	0.827	8	2.370	4.333	9
PS(15:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	793.95	16.588	48.333	9	5.423	11.206	9
PS(15:1(9Z)/0:0)	481.16	18.588	42.330	9	5.562	12.392	12
PS(15:1(9Z)/22:2(13Z,16Z))	799.61	0.003	0.003	3	0.013	0.016	6

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PS(15:1(9Z)/22:4(7Z,10Z,13Z,16Z))	795.41	0.020	0.022	4	0.035	0.048	3
PS(16:0/0:0)	497.31	8.253	23.970	9	5.907	14.433	11
PS(16:0/16:0)	735.08	0.814	1.845	9	5.571	10.996	10
PS(16:0/18:1(11Z))	761.13	0.971	1.600	7	4.034	8.469	8
PS(16:0/20:0)	791.87	162.053	485.304	9	3.989	8.564	10
PS(16:0/22:1(11Z))	817.67	0.711	1.852	12	3.467	8.883	11
PS(16:0/22:2(13Z,16Z))	815.31	4.161	8.664	7	4.711	8.333	7
PS(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	807.63	17.679	49.747	8	0.150	0.310	11
PS(16:1(9Z)/0:0)	494.32	1.076	2.322	8	2.991	5.302	8
PS(16:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	779.46	34.773	84.786	6	0.011	0.019	8
PS(16:1(9Z)/22:2(13Z,16Z))	813.50	3.084	6.891	5	0.009	0.015	4
PS(16:1(9Z)/22:4(7Z,10Z,13Z,16Z))	809.09	0.005	0.007	3	0.029	0.066	7
PS(16:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	805.35	11.054	34.920	10	5.093	11.368	9
PS(17:0/14:1(9Z))	719.72	1.687	3.632	8	7.171	14.210	10
PS(17:0/20:4(5Z,8Z,11Z,14Z))	797.57	0.935	1.778	9	3.553	5.509	8
PS(17:0/21:0)	819.81	0.017	0.032	4	1.506	2.399	7
PS(17:0/22:1(11Z))	830.90	0.400	0.881	11	3.079	6.904	10
PS(17:0/22:2(13Z,16Z))	828.89	17.808	46.522	7	1.385	3.589	10
PS(17:0/22:4(7Z,10Z,13Z,16Z))	825.84	1.687	3.150	7	6.191	16.784	13
PS(17:1(9Z)/0:0)	509.25	50.104	86.781	3	0.020	0.025	5
PS(17:1(9Z)/22:2(13Z,16Z))	827.69	0.031	0.059	5	0.024	0.026	6
PS(17:1(9Z)/22:4(7Z,10Z,13Z,16Z))	823.17	13.805	43.982	11	0.931	2.144	9
PS(17:2(9Z,12Z)/0:0)	507.32	9.950	27.513	10	6.898	15.157	9
PS(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z))	821.74	0.001	0.001	2	0.062	0.046	3
PS(18:0/0:0)	525.73	11.589	22.231	10	5.643	9.924	8
PS(18:0/12:0)	707.97	10.918	37.024	12	1.599	4.547	12
PS(18:0/18:1(9Z))	789.87	1.802	4.793	11	3.956	8.125	10
PS(18:0/20:4(5Z,8Z,11Z,14Z))	811.04	0.136	0.238	4	0.062	0.120	4
PS(18:0/22:1(11Z))	845.83	11.921	30.589	8	6.903	20.371	10

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PS(18:0/22:2(13Z,16Z))	843.43	0.291	0.447	5	3.027	7.061	9
PS(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	835.41	11.741	25.420	7	9.034	18.750	9
PS(18:1(9Z)/0:0)	523.22	46.424	120.982	9	89.882	240.798	11
PS(18:1(9Z)/18:1(9Z))	787.11	0.226	0.548	7	0.047	0.017	8
PS(18:1(9Z)/22:2(13Z,16Z))	840.97	0.237	0.422	10	2.000	6.037	10
PS(18:1(9Z)/22:4(7Z,10Z,13Z,16Z))	838.13	13.945	42.847	10	2.202	4.330	9
PS(18:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	832.87	14.393	42.209	10	3.382	9.863	11
PS(18:2(9Z,12Z)/0:0)	520.97	35.544	98.611	13	305.543	911.091	9
PS(18:2(9Z,12Z)/18:2(9Z,12Z))	783.43	0.977	2.232	9	2.949	5.459	9
PS(18:2(9Z,12Z)/22:2(13Z,16Z))	839.87	0.004	0.008	5	0.002	0.001	3
PS(18:3(6Z,9Z,12Z)/0:0)	518.90	24.421	69.571	9	5.038	9.695	5
PS(18:4(6Z,9Z,12Z,15Z)/0:0)	516.77	1.777	5.445	12	1.895	3.576	7
PS(19:0/0:0)	539.96	13.713	44.809	11	1.710	3.411	10
PS(19:0/21:0)	848.05	0.522	1.040	7	1.615	3.494	6
PS(19:0/22:0)	861.16	21.237	67.390	11	3.143	7.865	11
PS(19:0/22:1(11Z))	859.20	12.994	32.639	8	0.743	1.795	12
PS(19:0/22:2(13Z,16Z))	857.22	4.585	13.773	10	3.567	7.320	10
PS(19:0/22:4(7Z,10Z,13Z,16Z))	853.40	1.161	2.661	9	2.409	4.676	10
PS(19:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	850.75	0.518	0.765	6	1.764	4.276	10
PS(19:1(9Z)/0:0)	537.13	24.862	75.813	10	7.150	12.150	11
PS(19:1(9Z)/22:2(13Z,16Z))	855.39	4.596	11.222	6	0.015	0.028	8
PS(19:1(9Z)/22:4(7Z,10Z,13Z,16Z))	851.24	0.548	1.217	5	0.017	0.026	7
PS(20:0/0:0)	553.41	0.396	0.885	9	8.530	19.933	9
PS(20:0/22:1(11Z))	873.71	2.106	3.422	9	4.368	7.844	11
PS(20:0/22:2(13Z,16Z))	871.14	4.032	5.113	6	1.057	2.442	9
PS(20:0/22:4(7Z,10Z,13Z,16Z))	867.27	8.718	27.286	10	4.210	9.554	8
PS(20:1(11Z)/0:0)	550.66	13.324	39.480	9	3.039	6.632	8
PS(20:1(11Z)/22:2(13Z,16Z))	869.87	69.469	138.936	4	0.014	0.026	7
PS(20:1(11Z)/22:4(7Z,10Z,13Z,16Z))	864.92	0.513	1.092	8	1.581	3.699	7

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PS(20:2(11Z,14Z)/0:0)	548.71	14.673	39.436	8	2.673	4.847	8
PS(20:2(11Z,14Z)/22:4(7Z,10Z,13Z,16Z))	863.52	0.067	0.144	5	0.070	0.106	3
PS(20:3(8Z,11Z,14Z)/0:0)	547.21	0.012	0.022	5	0.010	0.013	4
PS(20:4(5Z,8Z,11Z,14Z)/0:0)	545.30	0.632	1.077	8	1.795	3.555	11
PS(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	542.68	1.175	2.547	9	1.743	3.577	10
PS(21:0(22:0)	890.27	17.791	46.132	9	5.824	12.928	11
PS(21:0(22:1(11Z))	887.23	0.795	1.237	8	4.384	6.017	6
PS(21:0(22:2(13Z,16Z))	886.01	0.015	0.032	5	0.008	0.013	9
PS(21:0(22:4(7Z,10Z,13Z,16Z))	881.51	0.006	0.006	6	0.032	0.058	6
PS(21:0(22:6(4Z,7Z,10Z,13Z,16Z,19Z))	877.03	21.475	62.548	10	3.874	8.378	11
PS(22:0(0:0)	581.67	5.742	13.122	9	2.343	6.665	9
PS(22:0(12:0)	763.49	49.430	85.605	3	0.020	0.031	7
PS(22:0(20:0)	875.54	122.401	367.169	9	0.003	0.003	5
PS(22:0(22:0)	904.27	19.779	41.841	5	2.977	5.175	9
PS(22:0(22:1(11Z))	901.35	1.179	2.572	12	4.321	10.800	9
PS(22:0(22:2(13Z,16Z))	899.03	6.641	14.948	8	0.221	0.445	8
PS(22:0(22:4(7Z,10Z,13Z,16Z))	895.48	12.260	36.824	11	61.685	182.481	11
PS(22:1(11Z)/0:0)	579.17	12.091	33.589	9	6.625	15.877	11
PS(22:1(11Z)/22:2(13Z,16Z))	897.05	0.006	0.001	3	0.289	0.453	3
PS(22:1(11Z)/22:4(7Z,10Z,13Z,16Z))	893.05	0.053	0.092	7	1.031	2.177	11
PS(22:2(13Z,16Z)/0:0)	576.53	1.683	2.940	6	2.988	7.055	10
PS(22:2(13Z,16Z)/22:4(7Z,10Z,13Z,16Z))	891.30	13.702	33.529	6	0.011	0.017	5
PS(22:4(7Z,10Z,13Z,16Z)/0:0)	572.96	6.590	15.633	8	7.168	15.826	8
PS(22:4(7Z,10Z,13Z,16Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	867.69Z)	5.870	13.784	10	7.243	12.949	8
PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	569.10	10.243	19.982	4	5.887	13.082	8
PS(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	1089.15Z,16Z,19Z))	15.548	40.094	8	4.092	9.654	11
PS(6:0(6:0)	455.36	1.092	2.237	8	4.007	7.778	13
PS(8:0(8:0)	511.24	0.611	1.435	10	3.446	7.101	11

*The lipid species identification is based on Lipidmaps database, used as a *.csv file for bioinformatic analyses with MZmine 2.9 program.

** A representative mass/charge ratio is presented (variations in m/z was reconciled by MZmine 2.9). Average standard non-normalized dataset is presented here. For some lipid species identified, standard error of mean could not be calculated due to lack of presence in all samples.

Table 4

Common lipid species between control and glaucomatous aqueous humor

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
NAPE(18:1(9Z)/16:1(9Z)/18:0)	982.02	0.393	0.445	7	15.314	3.745	6
PE(10:0/10:0)	523.85	0.108	0.183	9	4.480	1.196	8
PE(12:0/12:0)	578.96	0.327	0.485	7	20.702	5.203	6
PE(12:0/13:0)	592.94	0.283	0.807	10	5.004	2.250	9
PE(12:0/14:0)	607.16	0.230	0.408	10	8.652	3.843	9
PE(12:0/14:1(9Z))	604.84	0.147	0.317	7	3.784	1.312	13
PE(12:0/15:0)	621.85	16.320	48.772	9	6.821	2.404	7
PE(12:0/15:1(9Z))	619.04	16.414	48.589	9	21.046	6.740	8
PE(12:0/16:1(9Z))	633.11	0.463	1.373	9	8.101	2.074	9
PE(12:0/17:1(9Z))	646.91	0.867	1.874	5	322.678	181.660	8
PE(12:0/17:2(9Z,12Z))	645.18	25.608	61.944	6	13.918	2.531	7
PE(12:0/18:2(9Z,12Z))	659.39	17.460	51.824	9	9.672	4.286	10
PE(12:0/18:3(6Z,9Z,12Z))	657.41	0.031	0.051	3	8.600	2.976	6
PE(12:0/18:4(6Z,9Z,12Z,15Z))	654.91	22.319	58.307	7	8.595	2.492	5
PE(12:0/19:0)	677.53	0.019	0.028	5	11.912	2.628	4
PE(12:0/20:1(11Z))	689.67	42.689	79.976	4	29.109	11.348	4
PE(12:0/20:3(8Z,11Z,14Z))	685.05	0.159	0.317	7	8.177	2.933	11
PE(12:0/20:4(5Z,8Z,11Z,14Z))	683.19	23.112	60.751	7	19.401	6.775	6
PE(12:0/20:5(5Z,8Z,11Z,14Z,17Z))	681.04	0.252	0.515	8	6.128	2.848	6
PE(12:0/22:4(7Z,10Z,13Z,16Z))	711.01	16.823	53.012	10	4.484	2.499	11
PE(13:0/17:1(9Z))	661.42	1.309	1.452	3	21.457	7.355	3
PE(13:0/18:2(9Z,12Z))	673.13	26.699	64.594	6	6.330	2.270	8
PE(13:0/18:3(6Z,9Z,12Z))	670.70	26.381	64.520	6	3.391	1.683	10
PE(13:0/18:4(6Z,9Z,12Z,15Z))	668.64	0.526	1.066	7	7.379	3.114	8
PE(13:0/20:1(11Z))	702.80	0.249	0.565	7	23.794	9.178	8
PE(13:0/20:2(11Z,14Z))	701.28	16.750	52.158	10	19.506	4.921	6
PE(13:0/20:3(8Z,11Z,14Z))	698.73	20.925	58.222	8	10.127	3.509	11

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PE(13:0/20:4(5Z,8Z,11Z,14Z))	697.01	0.295	0.600	6	12.809	6.174	11
PE(13:0/20:5(5Z,8Z,11Z,14Z,17Z))	694.97	21.040	57.837	8	13.718	4.529	7
PE(13:0/22:2(13Z,16Z))	729.04	0.236	0.400	8	21.926	5.675	5
PE(13:0/22:4(7Z,10Z,13Z,16Z))	725.81	19.198	57.021	9	25.298	3.972	6
PE(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	722.00	15.917	51.167	11	7.759	3.660	7
PE(14:0/0:0)	425.22	0.249	0.466	9	9.153	3.416	8
PE(14:1(9Z)/0:0)	422.49	14.334	37.629	7	15.242	7.280	9
PE(14:1(9Z)/14:1(9Z))	631.57	0.033	0.065	5	22.889	6.255	7
PE(14:1(9Z)/20:4(5Z,8Z,11Z,14Z))	709.21	24.259	63.098	7	24.442	9.694	6
PE(14:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	707.68	56.067	95.783	3	0.126	0.041	5
PE(14:1(9Z)/22:4(7Z,10Z,13Z,16Z))	737.31	19.708	57.838	9	184.469	90.526	7
PE(15:0/0:0)	439.92	0.198	0.380	6	14.857	3.511	7
PE(15:0/15:0)	663.68	0.202	0.444	10	5.915	2.054	10
PE(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	723.60	28.426	69.611	6	4.521	2.011	5
PE(15:0/22:1(11Z))	759.63	0.414	0.824	4	0.923	0.386	7
PE(15:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	750.38	0.200	0.383	6	8.114	2.366	5
PE(15:1(9Z)/0:0)	437.35	13.505	36.268	8	3.605	0.650	6
PE(15:1(9Z)/22:2(13Z,16Z))	755.46	0.008	0.010	2	0.071	0.000	2
PE(15:1(9Z)/22:4(7Z,10Z,13Z,16Z))	751.63	0.976	1.555	4	25.386	11.048	6
PE(16:0/0:0)	453.26	11.954	35.530	9	3.529	0.910	8
PE(16:0/16:0)	691.36	0.126	0.186	9	167.619	74.217	6
PE(16:0/18:1(9Z))	717.71	0.124	0.200	8	12.584	4.883	12
PE(16:0/18:2(9Z,12Z))	715.37	0.767	0.776	3	0.147	0.035	3
PE(16:0/18:3(9Z,12Z,15Z))	713.21	0.276	0.564	7	12.653	4.261	9
PE(16:0/20:4(5Z,8Z,11Z,14Z))	739.07	0.315	0.540	4	102.423	55.374	9
PE(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	763.97	0.493	0.734	5	13.570	2.516	8
PE(16:1(9Z)/0:0)	450.68	21.570	47.373	5	19.296	5.901	8
PE(16:1(9Z)/16:1(9Z))	687.28	0.295	0.474	9	11.506	2.925	9
PE(16:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	735.91	29.264	70.609	6	0.047	0.017	4
PE(16:1(9Z)/22:2(13Z,16Z))	769.02	60.947	104.118	3	17.047	5.361	3

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PE(16:1(9Z)/22:4(7Z,10Z,13Z,16Z))	765.87	30.390	73.583	6	0.202	0.073	4
PE(17:0/14:1(9Z))	675.68	0.131	0.250	8	14.494	3.079	6
PE(17:0/20:0)	761.21	18.459	56.579	10	16.303	5.813	9
PE(17:0/20:4(5Z,8Z,11Z,14Z))	753.61	0.500	0.687	9	20.141	6.116	7
PE(17:0/22:0)	789.02	62.205	186.106	9	19.943	7.369	10
PE(17:0/22:1(11Z))	787.15	0.380	0.668	6	2.725	0.884	7
PE(17:0/22:2(13Z,16Z))	785.18	0.348	0.673	6	5.591	1.359	7
PE(17:0/22:4(7Z,10Z,13Z,16Z))	781.81	0.921	1.388	9	11.534	3.681	8
PE(17:1(9Z)/0:0)	464.71	0.185	0.350	8	6.163	1.406	5
PE(17:1(9Z)/22:2(13Z,16Z))	783.56	30.933	75.416	6	0.298	0.108	4
PE(17:1(9Z)/22:4(7Z,10Z,13Z,16Z))	779.06	0.004	0.006	2	9.687	3.551	8
PE(17:2(9Z,12Z)/0:0)	462.32	0.018	0.020	7	6.919	2.290	7
PE(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z))	777.71	0.002	0.001	2	0.163	0.057	4
PE(18:0/0:0)	481.44	0.216	0.313	7	8.163	2.539	3
PE(18:0/10:0)	635.66	0.058	0.084	6	5.015	1.602	11
PE(18:0/18:0)	747.98	16.534	52.994	11	12.553	1.723	4
PE(18:0/18:1(9Z))	745.61	0.412	0.805	4	5.266	1.639	4
PE(18:0/20:0)	775.28	0.634	1.659	9	186.743	107.624	9
PE(18:0/22:0)	803.28	0.236	0.312	10	15.228	4.133	6
PE(18:0/22:1(13Z))	801.87	48.453	93.791	4	10.863	5.592	7
PE(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	791.81	0.368	0.625	7	13.519	6.242	10
PE(18:1(9Z)/0:0)	478.50	14.127	39.848	8	4.067	1.312	6
PE(18:1(9Z)/18:2(9Z,12Z))	740.95	0.268	0.498	10	4.842	1.488	8
PE(18:1(9Z)/22:2(13Z,16Z))	796.90	0.099	0.169	8	4.327	1.114	6
PE(18:1(9Z)/22:4(7Z,10Z,13Z,16Z))	794.16	21.060	62.200	9	5.727	1.957	8
PE(18:2(9Z,12Z)/0:0)	477.02	0.528	1.264	6	4.675	2.511	10
PE(18:2(9Z,12Z)/22:2(13Z,16Z))	795.86	0.259	0.557	5	1.912	0.410	2
PE(18:3(6Z,9Z,12Z)/0:0)	475.01	12.621	37.217	9	9.447	2.588	7
PE(18:4(6Z,9Z,12Z,15Z)/0:0)	473.14	0.405	0.546	3	7.721	2.338	9
PE(19:0/0:0)	495.74	0.201	0.369	8	14.544	3.156	3

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PE(19:0/16:0)	733.19	0.414	0.571	8	8.692	3.863	10
PE(19:0/22:1(11Z))	814.99	0.596	0.829	6	38.991	17.638	8
PE(19:0/22:2(13Z,16Z))	813.46	76.689	171.427	5	8.734	2.084	4
PE(19:0/22:4(7Z,10Z,13Z,16Z))	808.97	0.478	1.227	8	25.247	8.903	5
PE(19:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	805.98	0.043	0.070	5	14.766	2.986	5
PE(19:1(9Z)/0:0)	493.49	14.783	41.043	8	29.666	13.434	10
PE(19:1(9Z)/22:4(7Z,10Z,13Z,16Z))	807.46	48.039	94.900	4	16.709	8.738	7
PE(20:0/0:0)	509.16	17.209	45.354	7	17.323	4.695	8
PE(20:0/18:1(9Z))	773.76	0.122	0.271	5	23.504	10.423	5
PE(20:0/18:4(6Z,9Z,12Z,15Z))	767.80	0.313	0.601	11	28.568	9.542	7
PE(20:0/20:2(11Z,14Z))	800.16	0.076	0.170	8	162.637	98.288	10
PE(20:0/22:0)	830.70	0.082	0.098	4	4.729	1.896	6
PE(20:0/22:1(13Z))	829.53	39.376	123.634	10	6.045	2.446	8
PE(20:0/22:2(13Z,16Z))	827.32	0.279	0.625	8	8.065	3.495	6
PE(20:0/22:4(7Z,10Z,13Z,16Z))	824.02	0.580	0.843	8	12.851	4.791	8
PE(20:0/24:1(15Z))	857.00	0.758	1.458	6	3.280	1.212	8
PE(20:1(11Z)/0:0)	507.06	0.004	0.004	6	3.488	1.096	8
PE(20:1(11Z)/22:2(13Z,16Z))	825.56	0.025	0.042	3	0.155	0.037	3
PE(20:1(11Z)/22:4(7Z,10Z,13Z,16Z))	821.36	0.303	0.567	10	14.272	4.291	8
PE(20:2(11Z,14Z)/0:0)	504.92	0.184	0.375	8	3.974	1.022	5
PE(20:2(11Z,14Z)/22:4(7Z,10Z,13Z,16Z))	819.37	0.002	0.001	3	19.078	9.249	6
PE(20:2(5Z,8Z)/18:0)	771.63	20.378	60.608	9	7.438	2.050	6
PE(20:3(8Z,11Z,14Z)/0:0)	503.09	0.291	0.474	5	0.304	0.078	8
PE(20:4(5Z,8Z,11Z,14Z)/0:0)	501.02	0.261	0.402	10	8.852	2.163	4
PE(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	498.99	15.159	41.412	8	128.071	56.383	5
PE(20:5(5Z,8Z,11Z,14Z,17Z)/22:5(7Z,10Z,13Z,16Z,19Z))	837.78	0.594	1.194	8	13.233	6.108	8
PE(21:0/22:0)	846.28	0.066	0.070	8	13.879	3.486	9
PE(21:0/22:1(11Z))	843.01	20.087	62.855	10	10.644	4.309	9
PE(21:0/22:2(13Z,16Z))	840.94	1.274	2.958	6	21.590	5.792	6
PE(21:0/22:4(7Z,10Z,13Z,16Z))	837.78	0.063	0.174	9	12.509	2.129	4

Lipid Species*	m/z	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/ μ g protein)	Std. Dev.	Donor Frequency
PE(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	833.18	20.265	61.978	10	18.894	5.912	10
PE(22:0/0:0)	538.14	18.205	47.906	7	7.693	2.855	9
PE(22:0/22:2(13Z,16Z))	855.37	0.358	0.738	6	4.773	1.734	10
PE(22:0/22:4(7Z,10Z,13Z,16Z))	851.60	0.530	0.783	10	14.149	3.264	8
PE(22:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	848.28	0.068	0.137	7	12.242	3.724	8
PE(22:0/24:1(15Z))	885.70	41.916	132.031	10	22.426	8.445	12
PE(22:1(11Z)/0:0)	535.35	11.708	37.992	11	18.256	5.722	7
PE(22:1(11Z)/22:2(13Z,16Z))	853.55	80.900	179.853	5	0.359	0.117	5
PE(22:1(11Z)/22:4(7Z,10Z,13Z,16Z))	849.31	1.419	1.984	3	0.250	0.060	4
PE(22:2(13Z,16Z)/0:0)	532.87	0.008	0.012	6	21.149	4.154	3
PE(22:4(7Z,10Z,13Z,16Z)/0:0)	528.67	0.098	0.117	5	9.333	3.256	8
PE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	525.52	0.253	0.441	6	3.379	1.231	5
PE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:4(7Z,10Z,13Z,16Z,19Z))	99.053	99.053	140.035	2	0.160	0.041	7
PE(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	723.504	22.368	65.446	9	6.881	3.067	9
PE(24:0/20:0)	859.43	0.261	0.338	10	27.241	8.803	7
PE(26:2(5Z,9Z)/26:2(5Z,9Z))	963.91	0.224	0.252	8	40.849	14.566	6
PE(6:0/6:0)	410.48	0.107	0.106	6	16.191	6.129	8
PE(8:0/8:0)	466.92	55.131	134.960	6	2.633	0.716	10
PE-NMe(11:0/11:0)	565.43	27.749	83.976	10	11.077	2.790	7
PE-NMe(14:0/14:0)	649.62	0.305	0.487	10	16.438	7.003	12
PE-NMe(16:0/16:0)	705.83	0.588	1.119	7	6.917	2.425	11
PE-NMe(16:0/18:1(9Z))	731.41	25.149	65.042	7	0.881	0.332	4
PE-NMe(18:1(9E)/18:1(9E))	758.00	20.386	59.385	9	13.268	4.494	7
PE-NMe(20:0/20:0)	817.96	24.251	68.068	8	79.995	39.241	10
PE-NMe2(16:0/16:0)	720.03	0.041	0.066	7	29.559	6.342	5

* The lipid species identification is based on Lipidmaps database, used as a *.csv file for bioinformatic analyses with MZmine 2.9 program.

** A representative mass/charge ratio is presented (variations in m/z was reconciled by MZmine 2.9). Average standard non-normalized dataset is presented here. For some lipid species identified, standard error of mean could not be calculated due to lack of presence in all samples.

Table 5

Common lipid species between control and glaucomatous aqueous humor

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PI(12:0/0:0)	516.51	0.469	0.802	9	0.042	0.057	6
PI(12:0/12:0)	697.84	0.172	0.326	10	0.132	0.180	4
PI(12:0/13:0)	712.38	0.929	1.798	6	0.249	0.514	7
PI(12:0/14:1(9Z))	724.47	0.509	1.230	6	0.016	0.037	6
PI(12:0/15:0)	739.76	0.948	2.571	8	0.207	0.307	4
PI(12:0/15:1(9Z))	738.42	0.640	1.487	10	0.263	0.530	7
PI(12:0/16:1(9Z))	752.53	1.253	1.565	8	0.092	0.262	9
PI(12:0/17:0)	768.55	0.500	1.195	8	0.113	0.267	7
PI(12:0/17:1(9Z))	766.08	0.166	0.319	5	0.018	0.046	8
PI(12:0/17:2(9Z,12Z))	763.79	0.973	1.456	7	0.034	0.061	5
PI(12:0/18:2(9Z,12Z))	778.44	0.338	0.827	11	0.073	0.110	7
PI(12:0/18:3(6Z,9Z,12Z))	776.31	1.090	1.219	6	0.036	0.068	5
PI(12:0/18:4(6Z,9Z,12Z,15Z))	774.82	9.96E-05	3.64E-05	2	0.003	0.002	3
PI(12:0/19:0)	797.12	0.565	1.120	9	0.028	0.041	6
PI(12:0/20:1(11Z))	807.84	0.389	0.679	7	0.011	0.010	4
PI(12:0/20:2(11Z,14Z))	806.13	0.398	0.687	6	0.006	0.004	4
PI(12:0/20:3(8Z,11Z,14Z))	804.40	0.709	1.711	7	0.028	0.061	6
PI(12:0/20:4(5Z,8Z,11Z,14Z))	801.56	1.649	2.314	2	0.072	0.123	3
PI(12:0/20:5(5Z,8Z,11Z,14Z,17Z))	800.40	0.004	0.008	6	0.535	1.328	7
PI(12:0/21:0)	824.57	9.953	19.772	6	1.574	3.825	6
PI(12:0/22:2(13Z,16Z))	833.98	0.005	0.008	5	0.008	0.004	2
PI(12:0/22:4(7Z,10Z,13Z,16Z))	830.57	0.144	0.296	10	1.109	2.870	7
PI(13:0/0:0)	530.53	0.844	1.610	4	0.109	0.188	5
PI(13:0/17:1(9Z))	780.45	0.687	0.737	6	0.049	0.064	4
PI(13:0/18:2(9Z,12Z))	792.24	0.607	1.304	6	0.004	0.006	3
PI(13:0/18:3(6Z,9Z,12Z))	789.90	0.527	1.215	8	0.003	0.002	7

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PI(13:0/18:4(6Z,9Z,12Z,15Z))	787.95	0.362	0.698	6	0.026	0.054	7
PI(13:0/20:1(11Z))	822.07	0.135	0.266	6	2.743	7.233	7
PI(13:0/20:2(11Z,14Z))	820.27	0.222	0.454	6	0.243	0.530	5
PI(13:0/20:3(8Z,11Z,14Z))	818.10	0.191	0.321	5	0.003	0.003	4
PI(13:0/20:4(5Z,8Z,11Z,14Z))	816.13	0.607	1.213	6	0.062	0.095	6
PI(13:0/20:5(5Z,8Z,11Z,14Z,17Z))	814.40	0.452	0.920	6	0.024	0.046	8
PI(13:0/22:0)	852.90	1.583	2.787	6	0.577	1.924	12
PI(13:0/22:1(11Z))	850.08	0.299	0.304	3	0.052	0.110	7
PI(13:0/22:2(13Z,16Z))	848.27	0.548	0.846	6	0.006	0.007	7
PI(13:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	840.47	0.218	0.269	12	0.058	0.110	8
PI(14:0/0:0)	544.13	1.484	1.967	5	1.970	5.066	7
PI(14:0/12:0)	726.85	0.329	0.729	5	0.080	0.168	5
PI(14:0/22:1(11Z))	864.65	0.076	0.131	4	0.016	0.018	2
PI(14:1(9Z)/0:0)	541.30	0.160	0.292	5	0.002	0.003	6
PI(14:1(9Z)/14:1(9Z))	750.24	0.719	1.142	8	0.010	0.017	6
PI(14:1(9Z)/20:4(5Z,8Z,11Z,14Z))	828.32	0.597	0.876	8	1.552	3.093	8
PI(14:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	826.62	0.001	0.001	2	0.016	0.017	5
PI(14:1(9Z)/22:4(7Z,10Z,13Z,16Z))	856.16	1.126	1.228	4	0.046	0.061	6
PI(15:0/0:0)	558.51	1.089	2.156	5	0.071	0.063	3
PI(15:0/13:0)	754.80	0.630	1.149	7	0.272	0.606	5
PI(15:0/20:3(8Z,11Z,14Z))	846.70	0.123	0.244	4	0.005	0.008	2
PI(15:0/20:5(5Z,8Z,11Z,14Z,17Z))	842.70	0.817	1.632	4	0.003	0.003	8
PI(15:0/22:0)	880.05	1.370	1.964	8	0.004	0.003	7
PI(15:0/22:1(11Z))	878.36	0.002	0.001	2	2.821	6.274	5
PI(15:0/22:2(13Z,16Z))	876.12	0.691	0.899	6	0.141	0.287	5
PI(15:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	869.41	1.659	2.556	6	0.005	0.003	6
PI(15:1(9Z)/0:0)	556.01	0.168	0.318	7	1.745	4.605	7
PI(15:1(9Z)/22:2(13Z,16Z))	874.75	1.233	2.128	3	0.004	0.004	4
PI(15:1(9Z)/22:4(7Z,10Z,13Z,16Z))	870.64	0.610	1.050	4	0.001	0.001	2

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PI(16:0/0:0)	572.02	1.038	2.116	10	0.308	0.756	7
PI(16:0/16:0)	810.36	0.874	1.208	7	0.178	0.347	4
PI(16:0/18:1(9Z))	836.92	0.700	0.744	6	0.228	0.473	8
PI(16:0/22:1(11Z))	892.44	0.198	0.297	5	0.033	0.070	6
PI(16:0/22:2(13Z,16Z))	890.67	1.089	1.742	6	0.036	0.053	9
PI(16:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	882.99	1.949	4.284	8	1.177	3.876	11
PI(16:1(9Z)/0:0)	569.31	2.682	4.593	3	0.009	0.015	8
PI(16:1(9Z)/20:5(5Z,8Z,11Z,14Z,17Z))	854.45	0.193	0.254	4	0.011	0.016	4
PI(16:1(9Z)/22:2(13Z,16Z))	888.51	1.059	1.751	3	0.004	0.005	5
PI(17:0/0:0)	586.35	0.178	0.353	4	0.099	0.213	8
PI(17:0/14:1(9Z))	794.91	0.643	0.983	5	1.051	2.565	6
PI(17:0/20:4(5Z,8Z,11Z,14Z))	872.80	0.643	1.202	7	0.172	0.275	8
PI(17:0/21:0)	894.90	0.924	1.625	7	0.028	0.038	8
PI(17:0/22:1(11Z))	906.48	0.566	0.908	7	1.062	1.988	8
PI(17:0/22:2(13Z,16Z))	904.01	0.657	1.307	7	0.009	0.015	5
PI(17:0/22:4(7Z,10Z,13Z,16Z))	900.86	0.595	1.256	6	0.249	0.725	10
PI(17:1(10Z)/0:0)	584.01	1.239	1.915	10	0.499	1.086	9
PI(17:1(9Z)/22:2(13Z,16Z))	902.46	0.931	1.265	3	0.012	0.021	5
PI(17:1(9Z)/22:4(7Z,10Z,13Z,16Z))	897.78	1.740	3.479	4	0.035	0.064	6
PI(17:2(9Z,12Z)/0:0)	581.88	0.115	0.246	7	0.134	0.276	6
PI(17:2(9Z,12Z)/22:4(7Z,10Z,13Z,16Z))	896.53	0.573	1.191	6	0.021	0.043	6
PI(18:0/0:0)	600.20	0.248	0.390	3	0.341	0.805	9
PI(18:0/12:0)	783.18	1.554	2.829	7	0.022	0.032	8
PI(18:0/18:0)	866.86	0.805	1.225	5	0.063	0.123	8
PI(18:0/20:4(5Z,8Z,11Z,14Z))	886.38	0.036	0.077	6	0.077	0.046	3
PI(18:0/22:1(11Z))	920.30	1.203	1.606	5	0.444	1.149	8
PI(18:0/22:2(13Z,16Z))	918.37	0.686	1.340	8	0.135	0.291	7
PI(18:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	910.80	1.464	3.707	8	0.246	0.585	8
PI(18:1(9Z)/0:0)	597.54	0.362	0.402	3	2.017	4.742	6

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PI(18:1(9Z)/22:2(13Z,16Z))	916.63	0.026	0.044	5	0.027	0.046	6
PI(18:1(9Z)/22:4(7Z,10Z,13Z,16Z))	912.70	1.810	3.651	5	4.093	8.146	4
PI(18:1(9Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	908.46	0.383	0.718	8	0.002	0.001	4
PI(18:2(9Z,12Z)/0:0)	596.14	0.528	0.875	5	0.063	0.081	7
PI(18:2(9Z,12Z)/22:2(13Z,16Z))	914.97	0.795	1.227	6	0.002	0.002	4
PI(18:3(6Z,9Z,12Z)/0:0)	594.17	0.818	1.260	10	0.033	0.069	5
PI(18:4(6Z,9Z,12Z,15Z)/0:0)	591.38	0.047	0.089	6	0.036	0.071	5
PI(19:0/0:0)	614.62	0.018	0.034	7	0.523	1.373	7
PI(19:0/21:0)	922.76	0.836	1.032	5	0.031	0.049	7
PI(19:0/22:0)	936.87	0.050	0.053	5	0.392	1.152	11
PI(19:0/22:1(11Z))	934.26	0.066	0.136	6	79.176	193.860	6
PI(19:0/22:2(13Z,16Z))	932.15	0.107	0.164	5	0.109	0.222	8
PI(19:0/22:4(7Z,10Z,13Z,16Z))	928.73	0.035	0.040	8	1.598	4.208	7
PI(19:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	924.89	0.025	0.041	8	0.012	0.021	4
PI(19:1(9Z)/0:0)	611.99	1.808	2.323	7	0.850	2.360	10
PI(19:1(9Z)/22:2(13Z,16Z))	930.51	0.850	1.567	4	0.107	0.227	6
PI(19:1(9Z)/22:4(7Z,10Z,13Z,16Z))	926.82	0.125	0.214	3	0.009	0.010	3
PI(20:0/0:0)	628.70	0.129	0.292	9	0.062	0.059	6
PI(20:0/22:1(11Z))	949.24	0.385	0.754	7	0.021	0.033	5
PI(20:0/22:2(13Z,16Z))	945.91	0.237	0.309	4	0.602	1.460	6
PI(20:0/22:4(7Z,10Z,13Z,16Z))	942.74	0.037	0.075	7	0.575	1.358	7
PI(20:1(11Z)/0:0)	625.95	1.264	1.553	4	0.901	2.533	8
PI(20:1(11Z)/22:2(13Z,16Z))	944.14	0.339	0.385	5	0.005	0.007	5
PI(20:1(11Z)/22:4(7Z,10Z,13Z,16Z))	940.12	0.269	0.512	8	0.154	0.271	5
PI(20:2(11Z,14Z)/0:0)	624.28	0.242	0.345	8	0.620	1.561	7
PI(20:3(8Z,11Z,14Z)/0:0)	622.61	1.140	2.421	5	0.006	0.010	5
PI(20:4(5Z,8Z,11Z,14Z)/0:0)	620.43	0.338	0.649	7	0.214	0.400	7
PI(20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	617.91	0.449	0.984	8	0.011	0.016	5
PI(21:0/0:0)	642.11	0.702	1.392	5	0.012	0.020	6

Lipid Species*	m/z**	Control			Glaucoma		
		Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency	Average normalized lipid amount (pmol per species/μg protein)	Std. Dev.	Donor Frequency
PI(21:0/22:0)	964.57	0.826	1.524	6	0.138	0.163	8
PI(21:0/22:1(11Z))	962.63	0.796	1.708	7	0.518	0.800	5
PI(21:0/22:2(13Z,16Z))	959.94	0.067	0.083	6	1.267	3.331	7
PI(21:0/22:4(7Z,10Z,13Z,16Z))	956.52	0.403	0.788	8	0.047	0.129	9
PI(21:0/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	952.51	0.259	0.745	10	2.915	6.635	6
PI(22:0/0:0)	656.83	0.484	0.701	7	0.005	0.005	3
PI(22:0/12:0)	838.44	0.854	1.419	4	0.005	0.007	3
PI(22:0/20:0)	951.05	0.702	1.580	6	0.005	0.005	4
PI(22:0/22:0)	978.93	0.155	0.287	9	0.024	0.038	7
PI(22:0/22:1(11Z))	976.55	0.599	0.691	8	0.083	0.152	6
PI(22:0/22:2(13Z,16Z))	974.32	0.404	0.815	9	0.016	0.024	4
PI(22:0/22:4(7Z,10Z,13Z,16Z))	971.24	0.063	0.093	5	0.044	0.061	6
PI(22:1(11Z)/0:0)	653.48	0.362	0.319	4	0.012	0.018	6
PI(22:1(11Z)/22:2(13Z,16Z))	973.07	0.080	0.119	4	0.040	0.061	7
PI(22:1(11Z)/22:4(7Z,10Z,13Z,16Z))	968.65	0.044	0.074	3	0.015	0.034	9
PI(22:2(13Z,16Z)/0:0)	651.97	0.001	0.001	4	0.271	0.569	7
PI(22:2(13Z,16Z)/22:4(7Z,10Z,13Z,16Z))	1449.58	0.429	0.607	2	0.009	0.015	4
PI(22:4(7Z,10Z,13Z,16Z)/0:0)	647.92	0.522	1.117	5	0.194	0.432	5
PI(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/0:0)	644.36	1.142	2.600	8	0.081	0.134	7
PI(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:4(7Z,10Z,13Z,16Z))	1024.58	0.008	0.011	2	0.007	0.012	6
PI(22:6(4Z,7Z,10Z,13Z,16Z,19Z)/22:6(4Z,7Z,10Z,13Z,16Z,19Z))	1026.58	0.774	1.257	5	0.005	0.004	6

* The lipid species identification is based on Lipidmaps database, used as a *.csv file for bioinformatic analyses with MZmine 2.9 program.

** A representative mass/charge ratio is presented (variations in m/z was reconciled by MZmine 2.9). Average standard non-normalized dataset is presented here. For some lipid species identified, standard error of mean could not be calculated due to lack of presence in all samples.

Table 6

Total Average Protein Normalized Phospholipids in the Aqueous Humor

	Phospholipids (pmol/ μ g protein)	
	Control	Glaucomatous
Phosphatidylcholines	201.427	195.625
Phosphatidylserines	3584.870	956.559
Phosphatidylethanolamines	1615.756	2897.872
Phosphatidylinositols	94.013	122.191
All Phospholipids (total)	5496.066	4172.247