Changes in the Lipid Profile of Aqueous Humor From Diabetic Cataract Patients

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Introduction

Diabetic cataract is one of the most common ocular complications of diabetes mellitus and is often associated with progressive visual loss. Diabetic patients are at increased risk of developing cataract, which tends to occur at an earlier age compared to the rest of the population.1,2 Although cataract surgery is very common and usually yields better visual results, it causes more vision-threatening complications in diabetic patients.3,4 With the increasing incidence of diabetes and aging, the occurrence rate of diabetic cataract will inevitably increase in the future. Therefore, in-depth exploration of the pathogenesis of diabetic cataracts and the search for nonsurgical approaches to prevent its formation and progression are fundamental, which has been a hot research topic for years. The pharmacological treatment conducted to reverse cataracts can have considerable health and economic impacts.5

Although several mechanisms have been proposed for determining the pathogenesis of diabetic cataract, it is yet to be completely understood. The abnormal activation of the polyol pathway, advanced glycation end products, and enhanced oxidative stress...
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Material and Methods

Subjects

This case-control study was approved by the ethical committee of Qili Hospital of Shandong University. The study protocol adhered to the tenets of the Declaration of Helsinki. Written informed consent forms were signed by each subject after the purpose and procedures of the study were completely explained. A definite previous medical history and the data on the current use of medications were collected as planned. The anonymity of all patients was preserved.

All patients had been diagnosed with type 2 diabetes mellitus (T2DM). T2DM patients without retinopathy were recruited as the diabetic group. Fundus grading in diabetic retinopathy was determined through dilated fundus photography examination conducted by two ophthalmologists, who were blinded to the patients’ clinical information. Healthy subjects who underwent routine cataract surgery were enrolled as the control group. Patients suffering from other ocular diseases, glaucoma, high myopia, retinal diseases or history of previous ocular surgery, or other severe systemic diseases and uncontrolled systemic hypertension were excluded from the study. Hyperlipidemia has been suggested as a high-risk factor for cataracts; therefore the subjects who had hyperlipidemia, as detected from a blood test, were excluded from the control group. Furthermore, subjects who used lipid-lowering drugs were also excluded. All subjects were consecutively enrolled from the Department of Ophthalmology at Qili Hospital of Shandong University between October 2020 and November 2021, and each subject received a comprehensive ophthalmic examination.

AH samples were collected at the time of the cataract surgery, as we have previously reported. Anterior chamber paracentesis was performed and 150–200 μL of AH was withdrawn using a tuberculin syringe attached to a 30-gauge needle. Blood samples were obtained in early morning on the first day of admission after overnight fasting. The serum of the blood sample was spun in a centrifuge at 4000 rpm for 20 minutes. The samples were transferred on dry ice and stored in Eppendorf tubes at −80°C until the assays were performed. The amounts of total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG), and fasting blood-glucose in the serum

are believed to be involved in the cataract formation in diabetic patients. These mechanisms can be therapeutic targets for diabetic cataract. According to some recent studies, several pharmacological substances possess anti-cataract ability, such as resveratrol, puerarin, and lanosterol; however, their effects remain unsatisfactory.

Lipids are a compound class in biological systems that play an essential role in numerous cellular processes. Hence, they are involved in the pathogenesis of many diseases, such as obesity, diabetes, cardiovascular disease, and chronic inflammation. Lipids are an essential component of the human lens. The degradation of membrane lipids significantly leads to cataractogenesis. More critically, lipid synthesis, especially for growth and repair, can also be compromised in cataract lenses. The systemic fatty acids present in the aqueous humor (AH) or drugs that decrease cholesterol synthesis can contribute to cataractogenesis. The idea that lipid changes in the lens can contribute to cataractogenesis has been extensively supported, enough to warrant further investigations.

AH is a transparent and viscous fluid that fills the anterior eye tissues. It not only supplies nutrients but also carries away metabolic wastes from avascular tissues in the eye. Normal AH contains diverse lipids. Several studies have been performed to determine the differential profiles of lipid species in the AH between normal subjects and glaucoma patients. Some lipids were found to be uniquely present in the glaucomatous AH and can contribute further insight into glaucoma pathology. Endogenous lipids in AH can act as modulators of intraocular pressure. Apart from glaucoma, distinct lipid profiles in AH have also been found in patients with conditions such as Fuchs endothelial corneal dystrophy and polypoidal choroidal vasculopathy. However, lipids in the AH of diabetic cataract patients remain less well studied, and no study has clarified how changes in AH lipids influence the development of diabetic cataract. The existence and the identification of differential lipids in AH can provide clues for discovering aberrant lipids enriched in the human lens.

Therefore our study primarily aims to assess the difference of lipid profiles in AH between diabetic and nondiabetic patients with cataracts. A lipidomic analysis based on liquid chromatography tandem mass spectrometry (LC-MS/MS) is conducted for the rapid characterization and absolute quantification of various lipid components in AH. Our secondary aim is to explore whether the lipid profile in AH correlates with diabetic cataract. A better understanding of the precise lipidic changes in AH that occur during diabetic cataract can provide new insights into the disease pathophysiology and yield novel treatment options.
were measured in the clinical laboratory of Qilu Hospital.

Method for Lipidomic Analysis

The lipidomic analysis was conducted by Applied Protein Technology (Shanghai, China), as a standard operation procedure. The methyl tert-butyl ether method was used for lipid extraction. In brief, the samples were homogenized with 200 μL methanol and 200 μL of water after being mixed with internal lipid standards. To this mixture, 800 μL of methyl tert-butyl ether was added. The mixture was subjected to ultrasound scanning for 30 minutes at 4°C and then kept still for half an hour at room temperature. After conducting centrifugation at 14,000 g at and 10°C for 15 minutes, the upper organic solvent layer was harvested. The lipid extracts were dried under nitrogen and re-dissolved in 200 μL of 90% isopropanol. After centrifugation at 14,000 g for 15 minutes, 3 μL of the sample was added. Solvent A was 60% acetonitrile and 40% water with 0.1% formic acid and 0.1 Mm ammonium formate. The initial mobile phase was 30% solvent B (90% isopropanol and 10% acetonitrile with 0.1% formic acid and 0.1 Mm ammonium formate) at a flow rate of 300 μL/min. It was held for two minutes and then linearly increased to 100% solvent B in 23 minutes and finally equilibrated at 5% solvent B for 10 minutes.

The lipid components were analyzed through an ultra-performance liquid chromatography–Orbitrap mass spectra (MS) system connected to a UHPLC Nexera LC-30A (Shimadzu, Kyoto, Japan). The MS were obtained in both positive and negative ion modes, respectively. The parameter settings were as follows: source temperature, 300°C; capillary temperature, 350°C; the scan range of the instruments’ mass-to-charge ratio (m/z), 200 to 1800; ion spray voltage, 3000V; and S-lens RF level, 50%.

The identification and quantification of the lipid components were identified simultaneously by using an automated search tool (LipidSearch software v.4.1; Thermo Fisher Scientific, Waltham, MA, USA). In the extracted ion features, only the variables with more than 50% of the nonzero measurement values in at least one group were kept. Both mass tolerance for the precursor and fragment were set to 5 ppm.

Statistical and Data Analysis

A statistical power analysis was performed using G*Power version 3.1.9.7 (Franz Faul, Universitat Kiel, Kiel, Germany) based on our preliminary data. The minimum numbers of samples required in the diabetic and control groups to achieve 80% power (type I error or false-positive rate at 0.05) were 18 and 26, respectively. A total of 51 samples (19 diabetic patients and 32 controls) were collected during the predetermined study period, and the actual power of this study was calculated as 81.8%.

Statistical analyses were performed using the statistical software SPSS version 20.0 software (SPSS Inc., Chicago, IL, USA). To identify the differentially expressed lipids between the two groups, a multivariate analysis was performed with SIMCA-P software (version 14.1; Umetrics, Umea, Sweden) after the Pareto-scaling process. The lipids with variable influence on projection (VIP) values > 1.0 and

| Table 1. Clinical Characteristics and Metabolic Parameters of the Study Subjects |
|--------------------------------|-----------------|-----------------|--------|
|                                | Diabetic Patients (Diabetic Group, n = 19) | Controls (Control Group, n = 32) | P Values |
| Age (y)                        | 57.63 ± 2.00     | 63.78 ± 1.81     | 0.071  |
| Sex                            |                 |                 | 0.403  |
| Male                           | 12              | 15              |        |
| Female                         | 7               | 17              |        |
| TCho (mmol/L)                  | 4.25 ± 0.35     | 4.51 ± 0.13     | 0.719  |
| HDL-C (mmol/L)                 | 1.24 ± 0.07     | 1.35 ± 0.34     | 0.806  |
| LDL-C (mmol/L)                 | 2.35 ± 0.26     | 2.64 ± 0.13     | 0.352  |
| TG (mmol/L)                    | 1.44 ± 0.29     | 1.09 ± 0.08     | 0.279  |
| FBG (mmol/L)                   | 6.40 ± 0.40     | 4.89 ± 0.12     | 0.006  |
| Hypertension, n (%)            | 12(63.2%)       | 9(28.1%)        | 0.030  |
| Duration of DM, y              | 9.05 ± 1.30     | —               | —      |

TCho, total cholesterol; FBG, fasting blood-glucose; DM, diabetes mellitus.

Data were expressed as mean ± standard errors.
P values < 0.05 were considered significant. The VIP and P values were obtained from the orthogonal partial least-squares-discriminant analysis (OPLS-DA) model and the two-tailed Student t-test conducted on the raw data, respectively. The Mann-Whitney test or Student t-test was used, as appropriate, to compare the independent samples between two groups. Fisher’s exact test or the χ² test was used for the comparison of categorical variables. The receiver operating characteristic (ROC) curves were performed to evaluate the diagnostic value of the target lipids in diabetic patients compared to the healthy subjects. All experimental data are presented as mean ± standard error. A P value < 0.05 was considered statistically significant.

Results

Subjects’ Characteristics

Lipidomic analyses were performed on 51 subjects: 19 diabetic cataract patients and 32 nondiabetic controls with age-related cataracts. The demographic

![Figure 1. Distribution and dynamic changes of detected lipid species in aqueous humor of the diabetic group and control group (A). Composition of lipid classes in both the control group (B) and diabetic group(C). Class abbreviations: PG, phosphatidylglycerol; PS, phosphatidylserine; PC, phosphatidyl choline; PA, phosphatidic acid; Cer, ceramide; CL, cardiolipin; DAG, diacylglyceride; LPC, lysophosphatidylcholine; LPE, lyso-phosphatidylethanolamine; LPG, lysophosphatidylglycerol; LPI, lysophosphatidylinositol; MGDG, mono-glycosyl diglycerides; PI, phosphatidylinositol; SM, sphingomyelin; ST, cholesterol; PE, phosphatidylethanolamine; PIP, phosphatidylinositol-4-monophosphate; DGDG, diglycosyl diglycerides.](image)
Figure 2. As is indicated in the bar chart, concentration of triglyceride (TG, A) was higher and ceramide-1-phosphates (CerP, B) was significantly lower in the aqueous humor of the diabetic group. *P < 0.05, **P < 0.01.

characteristics of all the subjects are summarized in Table 1. Significant differences were not detected in the gender, age, serum TG, total cholesterol, LDL-C, and HDL-C between the two groups. The diabetic patients had higher levels of fasting blood glucose and hypertension than the control subjects.

Lipid Class Analysis of AH
As determined by the LC-MS/MS analysis, the relative composition (%) of lipid classes in AH differed between the two groups. The vast majority of lipids in both groups were phosphatidylglycerol and there was no difference between the two groups (Fig. 1). However, the diabetic group showed a significant increase in the TG percentage, whereas the control group presented a higher composition of ceramide-1-phosphates (CerP) (Fig. 2).

Lipid Species Analysis of AH
In total, 639 lipids were reliably detected in both two groups: 131 TGs, 50 diacylglycerols (DGs), 87 phosphatidyl cholines (PCs), 37 phosphatidylethanolamines, 41 sphingomyelins, 21 sphingosidylserines, and other lipid species. Because OPLS-DA tends to overfit data, the model was validated and the lipids with VIP values > 1.0 and P values < 0.05 were considered significant. From the principal component analysis and OPLS-DA models, there was a notable degree of fit and predictive ability for the studied samples (Fig. 3), and 16 lipid species were determined that differentiated between the two groups. Among them, eight lipid species (i.e., three DGs, two TGs, one PC, one sterol lipid, and one phosphatidylinositol-4-monophosphate) were significantly increased, whereas the other eight lipid species (i.e., four DGs, two ceramides, one polyamide, and one phosphatidylserine) were significantly decreased in the diabetic group. The VIP scores and p values that discriminated between the two groups are shown in Table 2. The significantly changed lipids were displayed using a bubble map (Fig. 4). The absolute quantification results showed that the expression levels of four species of glycerolipids in the diabetic group increased more than twice compared to the control group, including three DGs in glycerolipids and one TG in glycerolipids (Fig. 5).

Potential Lipid Biomarkers in AH
To appraise the capability of these differently expressed lipids as potential diagnostic biomarkers, ROC analysis was performed for each lipid. Metabolites with an area under the curve (AUC) > 0.75 were reported to the ideal forecast classification for the individual subject among groups. The top two lipids based on the AUC scores were TG(42:6) (Fig. 6A, AUC = 0.985, P < 0.0001) and DG(24:2) (Fig. 6B, AUC = 0.944, P < 0.0001), which
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Figure 3. Principal component analysis (PCA) and OPLS-DA plot show clearly distinguished separation of the two groups.

Table 2. Lipidomic Differences in the Comparison of Diabetic Cataract and Control Subjects

| Lipid Group     | Class       | Fold Change | P Value | VIP | Direction of Change |
|-----------------|-------------|-------------|---------|-----|---------------------|
| CerP(d47:6)     | CerP        | 0.55        | 0.012   | 1.02| ↓                   |
| PA(40:3)        | PA          | 0.68        | 0.024   | 4.47| ↓                   |
| PC(32:0)        | PC          | 1.52        | 0.023   | 1.09| ↑                   |
| ST(m39:3)       | ST          | 1.05        | 0.041   | 1.02| ↑                   |
| DG(21:3)        | DG          | 2.86        | 0.044   | 1.27| ↑                   |
| DG(24:2)        | DG          | 2.87        | <0.001  | 2.84| ↑                   |
| DG(38:5e)       | DG          | 2.20        | <0.001  | 1.43| ↑                   |
| DG(21:5e)       | DG          | 0.71        | 0.001   | 3.21| ↓                   |
| PIP(12:0)       | PIP         | 1.33        | 0.049   | 6.47| ↑                   |
| PS(35:3e)       | PS          | 0.79        | 0.040   | 1.30| ↓                   |
| TG(52:4)        | TG          | 1.56        | 0.028   | 1.06| ↑                   |
| TG(42:6)        | TG          | 2.50        | <0.001  | 1.42| ↑                   |
| Cer(m44:3)      | Cer         | 0.48        | 0.009   | 1.69| ↓                   |
| DG(21:5e)       | DG          | 0.75        | 0.004   | 2.17| ↓                   |
| DG(32:1e)       | DG          | 0.94        | 0.045   | 1.70| ↓                   |
| DG(34:2)        | DG          | 0.95        | 0.039   | 1.17| ↓                   |

PA, phosphatidic acid; PC, phosphatidyl choline; ST, cholesterol; PS, phosphatidylserine; PIP, phosphatidylinositol-4-monophosphate.

indicated their high prediction capability for diabetic cataract.

Pearson correlation was used to further assess the relationship between the top two lipids and clinical parameters in diabetic cataract patients. The results showed that the expression levels of TG(42:6) in AH were positively related with the serum TG levels (Fig. 7).

Discussion

The detailed pathogenesis of diabetic cataract remains elusive. Previous literature reported that elevated glucose concentration and the loss of antioxidant activity in the lens initiate the development of diabetic cataract. Some have suggested that
hyperlipidemia and low HDL-C are related to an earlier onset and a higher incidence of diabetic cataract. In contrast to serum, AH is in direct contact with the lens surface and likely to be a more accurate reflection of the intraocular milieu. Thus we performed a LC-MS/MS analysis to determine the lipid profiles of AH from normal controls and patients with diabetic cataract. To the best of our knowledge, this is the first lipidomics study aiming to assess the difference in lipid compositions of AH between diabetic and nondiabetic patients with cataract.

The lipid class analysis in this study showed that an increased AH level of TG in the diabetic group. We performed further analysis of the lipid species and found that the expression levels of eight lipid species (including three DGs and two TGs) in AH were higher in the diabetic group than in the normal group. An ROC analysis was conducted to evaluate the diagnostic value of these differentially expressed lipid species. The results showed that only TG(42:6) and DG(24:2) were associated with diabetic cataract because they had AUC > 0.75, which is much higher than that reported by other lipids. Furthermore, the expression levels of TG(42:6) in AH were positively related with the serum TG levels. Undoubtedly, an elevated serum level of TG is a better predictor of cardiovascular disease, and TG lipolysis can lead to the production of lipids that are toxic to the surrounding tissue. The relationship between serum TG and cataract has been extensively studied. Li et al. reported that the serum LDL-C and TG were significantly higher in the age-related cataract group than in the control group and were demonstrated to be independent risk factors for age-related cataract. Paunksnis et al. reported a positive association between high TG levels and any cataract subtype among women aged 45 to 64 years. In addition, hyperlipidemia might be associated with the onset of cataract in patients with T2DM, and that diabetic cataracts might be accelerated by hyperlipidemia and low HDL-C in animal models. Our study also found that the serum TG concentration was higher in the diabetic group. However, no statistically significant difference was found between the two groups, and no significant association was observed between serum TG and cataract in diabetic group. Our study results differ from these previous results, which is attributable to the relatively small sample size. According to our results, the transport and oxidation of TGs and DGs were markedly impaired in the AH of diabetic patients. These differentially expressed TGs and DGs might contribute to the development of diabetic cataracts by increasing the formation of excess oxidants and reactive oxygen species in the lens. Further research needs to be performed to explore the clinical significance of elevated AH levels of TGs and DGs in diabetic patients.

We also observed eight reduced lipid species in the AH of diabetic patients. The two biggest drops of lipids were Cer(m44:3) and CerP(d47:6) in sphingolipids. Sphingolipids are important structural components of membranes, and play an equally important role in fundamental cellular processes as second messengers. Ceramide is the central molecule that regulates sphingolipid metabolism and has emerged as an essential node for lipid signaling. Previous studies have shown that increased cellular levels of ceramide accompany apoptosis and cell death in response to stress factors. At low concentrations in cell cultures, ceramides can reduce cell viability and increase apoptosis in lens epithelial cells, which suggests that ceramide has a key function in the development of age-related cataracts. As previously reported, the concentration of ceramides in the lens increase with age. In our study, the expression levels of Cer(m44:3) and CerP(d47:6) in the AH of diabetic patients were significantly lower compared to those in nondiabetic controls. This indicates the different pathogeneses between diabetic cataract and age-related cataract.

Recent reports have suggested that prolonged use of lipid-lowering drugs (e.g., statins) can predispose to cataract development. However, the pathogenic and
Figure 5. Scatter plots showing the distribution aqueous humor levels of TG(42:6) (A), DG(38:5e) (B), DG(24:2) (C), DG(21:3) (D) in diabetic and non-diabetic patients with cataract. *$P < 0.05$. ***$P < 0.001$.

The protective effects of lipid-lowering drugs on cataract are poorly documented and widely debated.35–37 To avoid its potential impact and how it might affect the results, the subjects who had used lipid-lowering drugs were excluded from the current study.

Figure 6. ROC analysis for TG (42:6) (A) and DG (24:2) (B) in distinguishing diabetic cataract patients from control subjects.
The expression level of TG (42:6) in aqueous humor was positively related with serum TG levels in diabetic cataract patients based on the Pearson’s correlation analysis.

The present study has several limitations. First, because this is a case-control and cross-sectional study, we cannot clarify the exact mechanisms underlying the association between the change in AH lipids and diabetic cataract. Second, the sample size was relatively small, and all of the subjects were Chinese, which might have limited the generalizability of the results. Third, whether diabetes treatment alters the expression levels of lipids in AH remains unclear. Therefore a multicenter study with a larger sample size should be conducted.

In summary, our study highlights the importance of the molecular interactions of lipid changes in AH and the development of diabetic cataracts. Lipids changes, especially the elevated levels of TG (42:6) and DG(24:2) in AH, could contribute to cataractogenesis. Novel therapy targeting lipids might be a potential therapeutic strategy for diabetic cataract. However, the role of these lipid species in the regulation of diabetic cataract remains unclear. A deeper understanding of the nature and extent of lipid changes in AH and the pathophysiological mechanisms behind the development of diabetic cataracts is required.

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