

# <sup>1</sup>H-NMR analysis of amino acid metabolism in aqueous humor of patients with cataract, according to diabetes status

Journal of International Medical Research  
0(0) 1–11

© The Author(s) 2020

Article reuse guidelines:

[sagepub.com/journals-permissions](https://sagepub.com/journals-permissions)

DOI: 10.1177/0300060520934658

[journals.sagepub.com/home/imr](https://journals.sagepub.com/home/imr)



Jing Jin<sup>1,2,3,4,\*</sup> , Qian Sun<sup>2,3,4,\*</sup>, Zan Wu<sup>5</sup>,  
Kun Liu<sup>2,3,4</sup>, Zhengyu Song<sup>6</sup> and Li Su<sup>2,3,4</sup>

## Abstract

**Objective:** To investigate the differences in amino acid metabolism in aqueous humor of patients with cataract, according to diabetes status, using a <sup>1</sup>H-nuclear magnetic resonance approach.

**Methods:** Aqueous humor samples from patients with age-related cataract, with or without diabetes, were collected during cataract surgery. All samples underwent nuclear magnetic resonance spectra analysis to characterize their metabolic function. Potential metabolic pathways were analyzed via MetaboAnalyst 3.0.

**Results:** This study included eight aqueous humor samples from patients with cataract and diabetes and eight aqueous humor samples from age- and sex-matched patients with cataract alone. Four metabolites were found to significantly differ in the aqueous humor of patients with cataract and diabetes, relative to patients with cataract alone; these metabolites were glucose (higher in patients with diabetes), valine, lysine, and tyrosine (all lower in patients with diabetes). Aminoacyl-tRNA biosynthesis was presumed to be involved in the metabolic differences observed in patients with cataract, according to diabetes status.

<sup>1</sup>Department of Ophthalmology, Shanghai Children's Medical Center, Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China

<sup>2</sup>Department of Ophthalmology, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China

<sup>3</sup>Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai, P. R. China

<sup>4</sup>Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai, P. R. China

<sup>5</sup>Department of Orthopedics, Shanghai Shibe Hospital of Jingan District, Shanghai, P. R. China

<sup>6</sup>Department of Ophthalmology, Shuguang Hospital, Shanghai University of Traditional Chinese Medicine, Shanghai, P. R. China

\*These authors contributed equally to this work.

## Corresponding author:

Li Su, Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, P.R. China.

Email: [sujilin615@163.com](mailto:sujilin615@163.com)



**Conclusions:** The amino acid metabolic profile in the aqueous humor differed among patients with cataract, according to diabetes status. Disturbance of amino acid metabolism in the aqueous humor may be related to cataract formation in patients with diabetes.

### Keywords

Aqueous humor, cataract formation, diabetes, amino acids, metabolite profiling, lysine, tyrosine, valine, glucose, transfer RNA

Date received: 21 December 2019; accepted: 26 May 2020

### Introduction

Cataracts are widely recognized as the leading cause of blindness worldwide.<sup>1</sup> Cataract-related blindness is expected to affect 40 million individuals globally by 2025.<sup>1,2</sup> Cataracts are formed via lens opacification, which reduces light transmission from the external environment to the retina, leading to vision impairment.<sup>3</sup> Diabetes is a class of metabolic diseases characterized by hyperglycemia; these diseases are associated with long-term damage and dysfunction of multiple organs and tissues, including the lens.<sup>4</sup> Patients with diabetes exhibit a five-fold increased risk of cataract development; they also experience earlier development of visually significant cataracts.<sup>5</sup> Severe cataracts can hinder observation of the retina and disturb retinal photocoagulation treatment for diabetic retinopathy, another diabetes-related vision-threatening complication; diabetes is also a risk factor for several cataract surgery-related complications.<sup>5</sup> Therefore, cataracts constitute a critical vision threat in patients with diabetes. Elucidation of the pathophysiology and mechanism of cataract development in patients with diabetes is important for its prevention and treatment.

The aqueous humor is transparent fluid in the anterior and posterior chamber around the lens. Aqueous humor is responsible for

supplying nutrients and antioxidants to the lens, as well as removing metabolic waste from the lens.<sup>2</sup> Aqueous humor is composed of proteins and small molecules, such as oxygen, glucose, amino acids, and lipids. The composition of aqueous humor depends on the nature of its production, as well as metabolic interchanges throughout its intra-ocular flow route. Previous studies have shown that the aqueous humor composition in pathological conditions differs from the composition in normal eyes.<sup>6-8</sup>

Metabolomics is considered an important tool for characterizing the composition of aqueous humor and revealing metabolic signatures of ocular diseases.<sup>9</sup> Metabolites have been studied in aging human lenses with cataract.<sup>10,11</sup> However, the amino acid metabolism of aqueous humor in patients with cataract and diabetes has not been fully elucidated; identification of metabolic signatures in aqueous humor in these patients may yield novel biomarkers and targets for new therapeutic treatments.

Here, we used <sup>1</sup>H-nuclear magnetic resonance (NMR) to explore differences in the amino acid metabolism of aqueous humor in patients with cataract, according to diabetes status; we also investigated presumed metabolic pathways involved in cataract formation in patients with diabetes. The findings may help to distinguish metabolic signatures in patients with cataract and diabetes.

## Methods

### Study design

This cross-sectional observational study regarding metabolite profiles of patients with cataract and diabetes was carried out at Shanghai General Hospital, Shanghai Jiao Tong University School of Medicine from June 2017 to May 2018. The study protocol was conducted in accordance with the tenets of the Declaration of Helsinki, and all procedures were approved by the Institutional Review Board of Shanghai General Hospital, Shanghai Jiao Tong University, School of Medicine (approval no. 2016KY115). Participants provided written informed consent before all study procedures.

### Sample collection

Patients with cataract, with or without diabetes, were recruited for this study and were divided into two groups: patients with cataract and type 2 diabetes, and age- and sex-matched patients with cataract alone. All included patients had a diagnosis of age-related cataract in the operated eye (nuclear hardness grade  $\geq 3$ ). Medical history and HbA1c levels were checked to determine diabetes status for all patients. Exclusion criteria were as follows: history of other ocular diseases, such as glaucoma, high myopia, uveitis, age-related macular degeneration, and diabetic retinopathy; history of ocular surgery or retinal photocoagulation; fasting blood glucose  $>8.5$  mmol/L; other chronic diseases (in addition to diabetes); and intake of systemic anti-metabolites, immunosuppressants, or corticosteroids.

All patients had fasted for 12 hours prior to the sample collection procedure. Aqueous humor samples were collected during cataract surgery by a single operator at Shanghai General Hospital. Briefly, the operated eye was rinsed twice with 5%

povidone iodine; subsequently, one or two drops of proparacaine hydrochloride 0.5% (Alcaine, Alcon, Ft. Worth, TX, USA) were applied twice to the operated eye. Approximately 100 to 150  $\mu\text{L}$  of aqueous humor were collected under a surgical microscope using a 1-mL tuberculin syringe and a 30-gauge blunt needle at the beginning of the surgical intervention. Aqueous humor samples were immediately transferred to 1.5-mL Eppendorf tubes (Eppendorf, Hamburg, Germany) and stored at  $-80^\circ\text{C}$  until analysis.

### $^1\text{H-NMR}$ measurement

$^1\text{H-NMR}$  metabolite profiling measurements were performed for patients with cataract, regardless of diabetes status, in a random order. Aliquots of aqueous humor (100  $\mu\text{L}$ ) were mixed with 400  $\mu\text{L}$  of phosphate buffer (0.2 M  $\text{Na}_2\text{HPO}_4/0.2$  M  $\text{NaH}_2\text{PO}_4$ , pH 7.4) to minimize variations. The aliquots were then centrifuged at  $12,000 \times g$  for 10 minutes at  $4^\circ\text{C}$  to pellet the precipitate. All NMR spectra were acquired at 298 K on an Avance NMR spectrometer (Bruker, Billerica, MA, USA) equipped with a cryogenic probe at 600.17 MHz for  $^1\text{H}$  observation. The NMR data of each sample were recorded using a solvent-suppressed one-dimensional  $^1\text{H}$  ZGPR pulse sequencer (RD-90°-ACQ). For aqueous humor samples,  $^1\text{H}$  NMR spectra were recorded via four dummy scans and 128 transient scans into 32,768 data points, using a spectral width of 20 ppm with a relaxation delay of 10.0 s and acquisition time of 2.73 s. All one-dimensional spectra were processed using an exponential function with 0.03-Hz line broadening and zero-filling to 65,536 data points. Additional two-dimensional pulsed field gradient CORrelation Spectroscopy and two-dimensional homonuclear Total Correlation Spectroscopy were performed with standard Bruker pulse programs on

selected samples to confirm the chemical shift assignments, as previously described.<sup>12</sup>

### **Multivariate statistical analysis**

The preprocessing protocol for all one-dimensional <sup>1</sup>H raw NMR spectra has been described previously.<sup>13</sup> The spectral region of each metabolite was integrated into a single bin. The resulting metabolite concentrations were then precisely calculated, in accordance with the integrals of standard samples. Subsequently, the concentration values were scaled to unit variance for principal component analysis by the SIMCA-P+12.0 software package (Umetrics, Umeå, Sweden). The principal component analysis and partial least squares discriminant analysis score plots were visualized with the first principal component (t[1]) and second principal component (t[2]). The partial least squares discriminant analysis and orthogonal projections to latent structures discriminant analysis score plots were visualized with the first principal component (t[1]) and first orthogonal component (to[1]). The parameters Q2 (cum), R2X (cum), and R2Y (cum) were computed to test the validity of the model against overfitting; R2X (cum) and R2Y (cum) were the total variations explained by the data, while Q2 (cum) was the cross-validated explained variation with increasing reliability as Q2 (cum) was approached.

To identify metabolites with significant contributions to the separations between study groups, the absolute values of correlation coefficients  $|r|$  (threshold  $> 0.5$ ) for assessing the relationships of variables with the first components of orthogonal projections to latent structures discriminant analysis models were extracted, as were the variable importance in the projection values (threshold  $> 1$ ). The correlation coefficient was used to characterize the first predictive component in the orthogonal

projections to latent structures discriminant analysis model. Additionally, the relative differences of metabolites between groups were calculated using normalized integrals, as follows:  $(I_A - I_B)/I_B$ , where  $I_A$  and  $I_B$  constitute the mean metabolite integrals corresponding to groups A and B for comparison in a single analysis model. Significant differences of intergroup variation were also evaluated with the nonparametric Wilcoxon matched-pairs signed-rank test, using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Results were considered statistically significant at  $p < 0.05$ .

### **Pathway analysis**

Metabolite profiles of aqueous humor in patients with cataract were compared according to diabetes status, using MetaboAnalyst 3.0, a web-based software derived from the KEGG metabolic pathways database.<sup>14</sup> Lists of metabolites (i.e., glucose, valine, lysine, and tyrosine) that had been identified from aqueous humor samples were imported into the software; the *Homo sapiens* pathway library was selected to explore potential related pathways. The related pathway names and impact values were displayed in the software output.

## **Results**

### **Study population**

This study included 16 participants: eight had age-related cataracts alone and eight had age-related cataracts with diabetes. The overall study population ranged in age from 51 to 85 years; patients with cataract alone ranged in from 51 to 85 years, and patients with cataract and diabetes ranged in age from 53 to 75 years. Table 1 presents the detailed demographic and clinical data of the two groups.

**Table 1.** Clinical and demographic characteristics of patients with cataract, according to diabetes status.

	Total (n = 16)	Cataract alone (n = 8)	Cataract with diabetes (n = 8)	p value
Age (years), mean $\pm$ standard deviation	71.94 $\pm$ 4.67	72.13 $\pm$ 4.32	71.75 $\pm$ 5.28	0.88
Sex (male), %	50	50	50	1.00
Body mass index (kg/m <sup>2</sup> ), median	23.45	22.96	23.93	0.43
Hypertension, n	4	0	4	0.02
Diabetes mellitus (years), mean $\pm$ standard deviation	3.19 $\pm$ 3.90	0	6.37 $\pm$ 3.01	0.00
Average HbA1c (%), mean ( $\pm$ standard deviation)	6.09 $\pm$ 1.01	5.16 $\pm$ 0.24	7.03 $\pm$ 0.38	0.00

### *Metabolic profiles of aqueous humor in patients with cataract, according to diabetes status*

Fifteen metabolites were detected in all samples: 3-methyl-2-oxovalerate, leucine, isoleucine, valine, lactate, alanine, lysine, glutamine, succinate, citrate, creatine, glucose, tyrosine, phenylalanine, and histidine. Among these 15 metabolites, the concentrations of four significantly differed in the aqueous humor of patients with cataract and diabetes, relative to patients with cataract alone; these metabolites were glucose (higher in patients with diabetes;  $p=0.02$ ), valine, lysine, and tyrosine (all lower in patients with diabetes;  $p=0.02$ ,  $p=0.03$ , and  $p=0.03$ , respectively) (Figure 1, Table 2).

### *Metabolic pathway analysis of aqueous humor in patients with cataract, according to diabetes status*

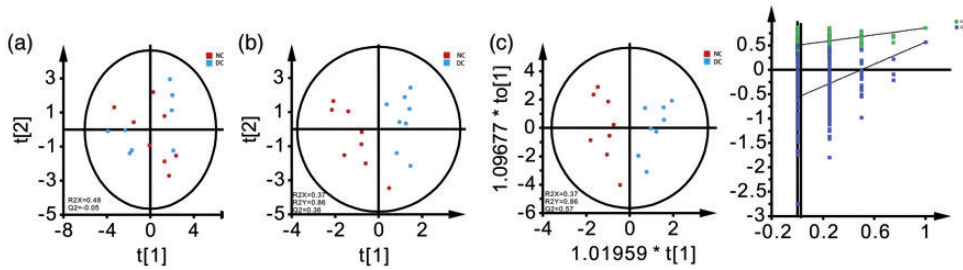
Metabolite profiles of aqueous humor were analyzed by MetaboAnalyst 3.0 software. The results of pathway impact analyses are shown in Figure 2; pathway analysis results are shown in Table 3. The aminoacyl-tRNA biosynthesis pathway was considered to be significantly implicated (impact value = 0.05).

## **Discussion**

In this study, using <sup>1</sup>H-NMR spectroscopy, we compared aqueous humor samples between age- and sex-matched patients with cataract, according to diabetes status. Our results showed that the concentrations of four metabolites (glucose, valine, lysine, and tyrosine) significantly differed in the aqueous humor of patients with cataract, according to diabetes status. These findings suggest that diabetes may disturb the amino acid metabolism of aqueous humor in patients with cataract and contribute to the progression of cataracts.

It has been reported that branched-chain amino acids, such as valine, play important roles in regulation of protein synthesis by activating mammalian target of rapamycin in pancreatic  $\beta$  cells.<sup>12</sup> Additionally, branched-chain amino acids are presumed to have positive effects on the regulation of glucose homeostasis;<sup>12</sup> elevated blood levels of branched-chain amino acids may be associated with insulin resistance.<sup>15</sup> Thus, the comparatively lower level of valine in the aqueous humor of patients with cataract and diabetes in our study may have arisen from a disorder of glucose metabolism and insulin secretion.

A previous study revealed that advanced glycation endproducts were important initiators of diabetic complications; moreover,



**Figure 1.** Metabolite profiling analyses of patients with cataract, according to diabetes status. (a) principal component analysis scatter plot, (b) partial least squares discriminant analysis scatter plot, (c) orthogonal projections to latent structures discriminant analysis scatter plot, and d) validation plot. In panel d, Q2 and R2 represent quality of fitted model. Q2 (cum) value greater than all fitted Q2 values in permuted tests indicated stable models with good fitness and excellent prediction abilities.

DC, patients with cataract and diabetes; NC, patients with cataract alone; R2, variation explained by fitted model; Q2, cross-validated explained variation in 7-round cross validation and permutation tests.

**Table 2.** Differences in metabolite integrals in aqueous humor of patients with cataract, according to diabetes status.

Metabolite	% change, DC vs. NC	r	VIP	p value
Valine	-19.8	0.45	1.37	0.02
Glucose	+70.6	0.56	1.47	0.02
Lysine	-29.9	0.53	1.29	0.03
Tyrosine	-10.9	0.61	1.54	0.03

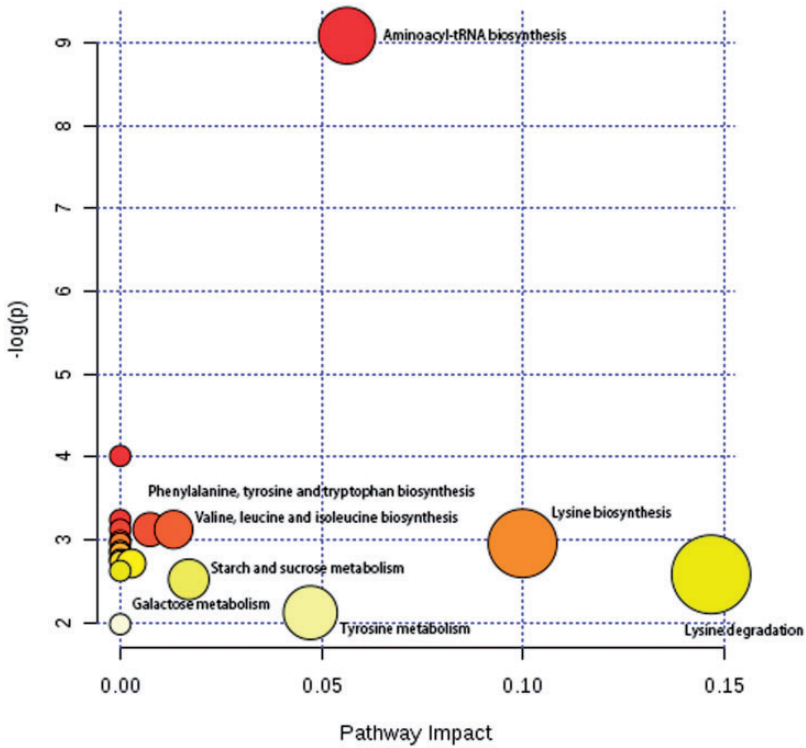
DC, patients with cataract and diabetes; NC, patients with cataract alone; VIP, variable importance in the projection.

enhanced reactive oxygen species generation induced by advanced glycation end-product interactions with the receptor for advanced glycation endproducts appeared to be an early histopathological hallmark of diabetes-related disorders.<sup>16</sup> Lysine has been proposed to play a role in advanced glycation endproduct modifications;<sup>17,18</sup> therefore, we suspect that the difference in lysine concentrations in the aqueous humor of patients with cataract, according to diabetes status, may have been associated with regulation of advanced glycation endproducts and reactive oxygen species.

Insulin resistance plays a central role in type 2 diabetes and its complications. A recent study showed that tyrosine phosphatases could dephosphorylate the insulin

receptor; therefore, the insulin receptor has been recognized as a potential therapeutic target.<sup>19</sup> In the present study, we found that the tyrosine content significantly varied in patients with cataract, according to diabetes status; the above-mentioned mechanisms may explain this difference.

Previous studies have demonstrated that the serum levels of valine, tyrosine, lysine, and other amino acids were elevated in rat models of diabetes.<sup>20,21</sup> Elevated levels of valine and tyrosine were also observed in patients with type 2 diabetes, compared with healthy controls.<sup>22</sup> These results were inconsistent with the changes we observed in aqueous humor samples from patients with cataract. We speculate that these discrepancies may be due to the natural



**Figure 2.** Pathway impact analysis of patients with cataract, according to diabetes status, by Metaboanalyst 3.0.

DC, patients with cataract and diabetes; NC, patients with cataract alone.

blood–retinal barrier and blood–aqueous barrier; they may also have occurred because aqueous humor metabolism depends more on the intraocular microenvironment compared with serum metabolism.<sup>23</sup> Therefore, further studies with larger numbers of patients are needed to explore the possible metabolic differences between serum and aqueous humor, and to determine whether these differences influence cataract formation and progression.

Because the four significantly altered metabolites (glucose, valine, lysine, and tyrosine) in patients with cataract and diabetes are closely related to the citric acid cycle, we suspect that elevated glucose may trigger metabolic disorders by

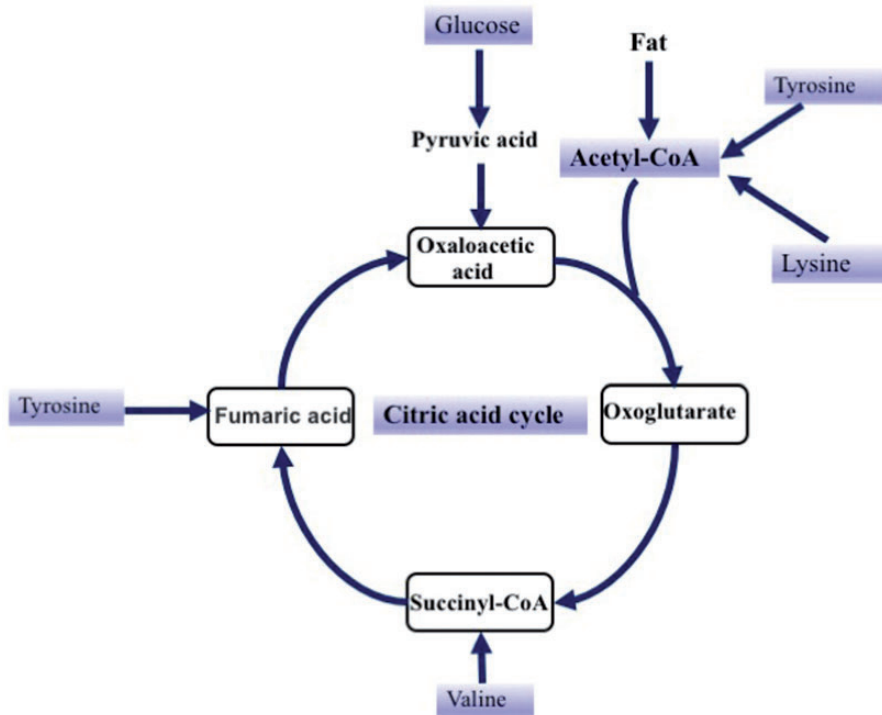
producing more oxaloacetic acid, thus leading to enhanced consumption of tyrosine, lysine, and valine.<sup>24</sup> This process may produce oxidative stress and accelerate cataract formation. Figure 3 shows the possible influences of metabolites on the citric acid cycle in the aqueous humor of patients with cataract and diabetes.

The metabolomic compositions of aqueous humor in human ocular diseases have been studied by using two major analytical platforms: high-resolution NMR spectroscopy and liquid or gas chromatography with mass spectrometry detection.<sup>25–30</sup> Using a liquid chromatography–mass spectrometry system, Pietrowska et al.<sup>26</sup> showed that several antioxidants (i.e., methyltetrahydrofolic acid, taurine, niacinamide, xanthine, and

**Table 3.** Pathway analysis of patients with cataract, according to diabetes status.

Pathway	Raw p	−log (p)	Holm p	FDR	Impact
Aminoacyl-tRNA biosynthesis	0.00	9.08	0.01	0.01	0.05
Biotin metabolism	0.02	4.01		0.38	0
Thiamine metabolism	0.04	3.24		0.38	0
Pantothenate and CoA biosynthesis	0.04	3.12		0.38	0
Phenylalanine, tyrosine, and tryptophan biosynthesis	0.04	3.12		0.38	0.01
Valine, leucine, and isoleucine biosynthesis	0.04	3.12		0.38	0.01
Glycolysis or gluconeogenesis	0.05	2.98		0.38	0
Pentose phosphate pathway	0.05	2.95		0.38	0
Lysine biosynthesis	0.05	2.95		0.38	0.10
Propanoate metabolism	0.05	2.87		0.38	0
Ubiquinone and other terpenoid-quinone biosynthesis	0.06	2.83		0.38	0
Nitrogen metabolism	0.06	2.76		0.38	0
Valine, leucine, and isoleucine degradation	0.06	2.74		0.38	0
Galactose metabolism	0.07	2.71		0.38	0.01
Phenylalanine metabolism	0.07	2.62		0.38	0
Lysine degradation	0.08	2.58		0.38	0.15
Starch and sucrose metabolism	0.08	2.52		0.38	0.02
Tyrosine metabolism	0.12	2.12		0.54	0.05
Amino sugar and nucleotide sugar metabolism	0.14	1.98		0.58	0

DC, patients with cataract and diabetes; NC, patients with cataract alone.



**Figure 3.** Influence of metabolites on citric acid cycle in aqueous humor of patients with cataract and diabetes.

CoA, coenzyme A.



uric acid) were reduced in aqueous humor of patients with diabetes. However, some amino acids (i.e., phenylalanine, leucine, and valine) were elevated in patients with cataract and diabetes, which differed from our findings. Using gas chromatography-time-of-flight mass spectrometry technology, Yao et al.<sup>31</sup> demonstrated that three pathways (i.e., fatty acid biosynthesis, fatty acid metabolism, and linoleic acid metabolism) were the most significantly influenced pathways, which suggests that these pathways play key roles in the formation of cataracts. Although a definitive explanation for the apparent discrepancies among these studies has not been established, the discrepancies may be related to differences in methodology (mass spectrometry vs. NMR) and sample size. Future studies in patients with cataract, using different analytical platforms and larger numbers of patients, may more precisely elucidate the sources of these differences. Overall, the findings of these studies suggest that diabetes disturbs amino acid metabolism in aqueous humor of patients with cataract.

The greatest limitation of our study was its small number of patients; the study may have been underpowered to detect smaller differences in aqueous humor metabolite profiles of patients with cataract, according to diabetes status. Additionally, owing to the cross-sectional nature of this study, we could not assess dynamic alterations in aqueous humor metabolite profiles during the course of cataract progression. This limitation may be addressed by future longitudinal studies with larger numbers of patients. Despite its limitations, this study demonstrated the effect of diabetes on amino acid metabolism in aqueous humor of patients with cataract, using <sup>1</sup>H-NMR spectroscopy.

## Conclusions

The amino acid metabolic profile differed in the aqueous humor of patients with

cataract, according to diabetes status. Disturbances in amino acid metabolism in aqueous humor may be related to cataract formation in patients with diabetes. These results may provide new insights for metabolism studies in patients with cataract, as well as potential therapeutic targets.

## Acknowledgment

We thank Professor Xia Liu for providing technical assistance in this study.


## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

## Funding

This study was funded by the National Key Research and Development Program of China (Grant No. 2016YFC0904800), National Natural Science Foundation of China (Grant No. 81800799), Projects of Shanghai Science and Technology Commission (Grant No. 19401932200), and Projects of Shanghai Municipal Health Commission (Grant No. 20194Y0268).

## ORCID iD

Jing Jin  <https://orcid.org/0000-0003-4905-0677>

## References

1. West S. Epidemiology of cataract: accomplishments over 25 years and future directions. *Ophthalmic Epidemiol* 2007; 14: 173–178.
2. Yanshole VV, Snytnikova OA, Kiryutin AS, et al. Metabolomics of the rat lens: a combined LC-MS and NMR study. *Exp Eye Res* 2014; 125: 71–78.
3. Ji Y, Rao J, Rong X, et al. Metabolic characterization of human aqueous humor in relation to high myopia. *Exp Eye Res* 2017; 159: 147–155.
4. Wiemer NG, Dubbelman M, Hermans EA, et al. Changes in the internal structure of the

- human crystalline lens with diabetes mellitus type 1 and type 2. *Ophthalmology* 2008; 115: 2017–2023.
5. Peterson SR, Silva PA, Murtha TJ, et al. Cataract surgery in patients with diabetes: management strategies. *Semin Ophthalmol* 2018; 33: 75–82.
  6. Han G, Wei P, He M, et al. Glucose metabolic characterization of human aqueous humor in relation to wet age-related macular degeneration. *Invest Ophthalmol Vis Sci* 2020; 61: 49.
  7. Jin H, Zhu B, Liu X, et al. Metabolic characterization of diabetic retinopathy: an <sup>1</sup>H-NMR-based metabolomic approach using human aqueous humor. *J Pharm Biomed Anal* 2019; 74: 414–421.
  8. Myer C, Perez J, Abdelrahman L, et al. Differentiation of soluble aqueous humor metabolites in primary open angle glaucoma and controls. *Exp Eye Res* 2020; 194: 108024.
  9. Chen L, Gao Y, Wang LZ, et al. Recent advances in the applications of metabolomics in eye research. *Anal Chim Acta* 2018; 1037: 28–40.
  10. Tamara SO, Yanshole LV, Yanshole VV, et al. Spatial distribution of metabolites in the human lens. *Exp Eye Res* 2016; 143: 68–74.
  11. Tsentelovich YP, Verkhovod TD, Yanshole VV, et al. Metabolomic composition of normal aged and cataractous human lenses. *Exp Eye Res* 2015; 134: 15–23.
  12. Kolanu BR, Boddula V, Vadakedath S, et al. Amino acid (leucine) chromatography: a study of branched-chain aminoaciduria in type 2 diabetes. *Cureus* 2017; 9: e1091.
  13. Liu X, Zhu W, Guan S, et al. Metabolomic analysis of anti-hypoxia and anti-anxiety effects of Fu Fang Jin Jing oral liquid. *PLoS One* 2013; 8: e78281.
  14. Xia J, Sinelnikov IV, Han B, et al. MetaboAnalyst 3.0—making metabolomics more meaningful. *Nucleic Acids Res* 2015; 43: W251–W257.
  15. Lu J, Xie G, Jia W, et al. Insulin resistance and the metabolism of branched-chain amino acids. *Front Med* 2013; 7: 53–59.
  16. Fokkens BT, Mulder DJ, Schalkwijk CG, et al. Vitreous advanced glycation endproducts and alpha-dicarbonyls in retinal detachment patients with type 2 diabetes mellitus and non-diabetic controls. *PLoS One* 2017; 12: e0173379.
  17. Ahmed MU, Thorpe SR and Baynes JW. Identification of N epsilon-carboxymethyllysine as a degradation product of fructoselysine in glycated protein. *J Biol Chem* 1986; 261: 4889–4894.
  18. Lal S, Kappler F, Walker M, et al. Quantitation of 3-deoxyglucosone levels in human plasma. *Arch Biochem Biophys* 1997; 342: 254–260.
  19. Stanford SM, Aleshin AE, Zhang V, et al. Diabetes reversal by inhibition of the low-molecular-weight tyrosine phosphatase. *Nat Chem Biol* 2017; 13: 624–632.
  20. Lin X, Zhao L, Tang S, et al. Metabolic effects of basic fibroblast growth factor in streptozotocin-induced diabetic rats: a (1)H NMR-based metabolomics investigation. *Sci Rep* 2016; 6: 36474.
  21. Park S, Park JY, Lee JH, et al. Plasma levels of lysine, tyrosine, and valine during pregnancy are independent risk factors of insulin resistance and gestational diabetes. *Metab Syndr Relat Disord* 2015; 13: 64–70.
  22. Newgard CB. Interplay between lipids and branched-chain amino acids in development of insulin resistance. *Cell Metab* 2012; 15: 606–614.
  23. Jahnke K, Wagner T, Bechrakis NE, et al. Pharmacokinetics and efficacy of ifosfamide or trofosfamide in patients with intraocular lymphoma. *Ann Oncol* 2005; 16: 1974–1978.
  24. Sivanesan S, Taylor A, Zhang J, et al. Betaine and choline improve lipid homeostasis in obesity by participation in mitochondrial oxidative demethylation. *Front Nutr* 2018; 5: 61.
  25. Barbas-Bernardos C, Armitage EG, García A, et al. Looking into aqueous humor through metabolomics spectacles—exploring its metabolic characteristics in relation to myopia. *J Pharm Biomed Anal* 2016; 127: 18–25.
  26. Pietrowska K, Dmuchowska DA, Krasnicki P, et al. An exploratory LC-MS-based metabolomics study reveals differences in aqueous humor composition between

- diabetic and non-diabetic patients with cataract. *Electrophoresis* 2018; 39: 1233–1240.
27. Pietrowska K, Dmuchowska DA, Samczuk P, et al. LC-MS-based metabolic fingerprinting of aqueous humor. *J Anal Methods Chem* 2017; 2017: 6745932.
  28. Snytnikova OA, Khlichkina AA, Yanshole LV, et al. Metabolomics of the human aqueous humor. *Metabolomics* 2017; 13: 5.
  29. Kunikata H, Ida T, Sato K, et al. Metabolomic profiling of reactive persulfides and polysulfides in the aqueous and vitreous humors. *Sci Rep* 2017; 7: 41984.
  30. Chen XL, Chen YH, Wang L, et al. Metabolomics of the aqueous humor in patients with primary congenital glaucoma. *Mol Vis* 2019; 25: 489–501.
  31. Yao YR, Wang HM, Zhu BJ, et al. Aqueous humor metabolomic profiles in association with diabetic mellitus. *Int J Clin Exp Pathol* 2018; 11: 3479–3486.