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ORIGINAL RESEARCH

Metabolic Deficiencies in the Vitreous of Diabetic Retinopathy: Exploring the Potential of Dietary Supplementation to Address Key Metabolite Imbalances

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Purpose: This study aimed to investigate the metabolic alterations in the vitreous humor of patients with diabetes across different stages of diabetic retinopathy (DR) and explore potential dietary interventions to mitigate these changes.

Patients and Methods: Vitreous samples were collected from 23 patients undergoing vitrectomy and grouped into controls, diabetic without DR, non-proliferative DR (NPDR), and proliferative DR (PDR). Metabolomic analysis was performed using mass spectrometry, focusing on identifying significantly altered metabolites.

Results: A total of 82 features were identified, of which several were significantly reduced in DR compared to controls. Ascorbate and taurine were notably lower in NPDR ($p < 0.01$), while choline and N-acetylaspartic acid were significantly reduced in NPDR and PDR ($p < 0.01$). Tagatose was significantly reduced in PDR ($p < 0.05$), while stachydrine and serine displayed biphasic trends, decreasing in NPDR ($p < 0.001$; $p < 0.05$) but increasing in PDR ($p < 0.05$).

Conclusion: The results suggest that key metabolites involved in antioxidant defense, membrane integrity, and neuronal function are disrupted in the vitreous humor of patients with DR. Dietary supplementation targeting these metabolic deficiencies, such as increased intake of ascorbate, taurine, serine, choline, and stachydrine, may offer adjunctive support in managing DR progression.

Keywords: diabetic retinopathy, metabolomics, dietary supplementation, vitreous humor, oxidative stress, retinal health

Introduction

Diabetes mellitus (DM) is a major medical and societal challenge due to the rapid increase in prevalence of type 2 DM and devastating late complications.[1,2] Globally, adults living with diabetes are estimated to have increased from 108 million in 1980 to 463 million in 2019, leading to an increased need for treatment of diabetic complications.[2,3] Among the most severe and common diabetic late complications is diabetic retinopathy (DR).[4,5] DR is the leading cause of vision loss in the working age population and in developed countries, and estimates show that approximately one-third of all DM cases develop retinopathy.[5–10] It is characterized by retinal microaneurysms, exudates, dot and blot hemorrhages, neovascularizations and diabetic macular edema.[4] In newly diagnosed type 2 DM, a large proportion of patients already have some degree of DR.[10,11]

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As a chronic complication of diabetes mellitus, DR is driven by a complex interplay of metabolic, vascular and neurodegenerative disturbances that inflict significant damage to the retinal microvasculature.[12–14] Despite advancements in our understanding of the vascular aspects of DR, the multifaceted molecular mechanisms that drive its onset and progression remain incompletely understood.[12]

The past decade has witnessed the emergence of metabolomics as an invaluable tool for deciphering the intricate metabolic alterations associated with various diseases.[15] The vitreous humor, residing in the posterior segment of the eye, serves as a dynamic repository of bioactive molecules that can offer insights into localized metabolic changes of the retina.[16] By probing the metabolome of the vitreous, we can unravel the network of biochemical pathways implicated in the pathogenesis of DR, furthering the potential for new therapeutic strategies.[17,18]

Several studies have investigated vitreous metabolomics in patients with PDR compared to controls, as well as plasma and serum metabolomes across different stages of DR.[19–25] However, to our knowledge, no studies have explored vitreous metabolomics across different stages of DR. In this case series study, we sought to investigate the vitreous metabolomes of patients with diabetes, but without DR (DIA), patients with non-proliferative DR (NPDR) and patients with proliferative DR (PDR), compared against control subjects (CTRL).[26] Our objective was to broaden our understanding of the metabolic changes that underlie the pathophysiology of DR. Using state-of-the-art mass spectrometry-based metabolomic techniques, we intended to identify distinct metabolites and pathways that exhibit aberrations in the vitreous of DR patients.[17,18]

Of particular interest is the prospect of nutritional supplementation as an adjunctive strategy to mitigate the metabolic disturbances driving DR.[27] Recent research has begun to illuminate the potential of specific nutrients, antioxidants, and bioactive compounds in modulating metabolic pathways implicated in DR.[28] By investigating the vitreous metabolomic data, we aspired to pinpoint novel interventional targets that could ameliorate metabolic imbalances and mitigate retinal damage in patients that are at risk for developing DR.[29]

Methods and Materials

Ethics

All patients provided written informed consent, and the study was approved by the Regional Committees for Medical and Health Research Ethics in Norway (REK no. 8050), in accordance with the Declaration of Helsinki.

Patients and Sample Collection

Patients scheduled for therapeutic pars plana vitrectomy (PPV) were prospectively enrolled from the Department of Ophthalmology, Oslo University Hospital (OUH), Oslo, Norway, and the Eye Hospital, University Medical Centre, Ljubljana, Slovenia. Recruitment occurred between February and August 2021. All participants underwent a clinical examination, including medication review, at the time of inclusion.

Inclusion criteria were patients aged ≥50 years undergoing therapeutic PPV. Exclusion criteria included a history of uveitis, retinal vascular occlusion, advanced glaucoma, intraocular surgery within the preceding three months (excluding cataract surgery), ongoing systemic infections, active systemic inflammatory disease, or active vitreous hemorrhage.

Patients were categorized into four groups: non-diabetic controls (CTRL; n = 6), diabetics without diabetic retinopathy (DIA; n = 5), non-proliferative diabetic retinopathy (NPDR; n = 5), and proliferative diabetic retinopathy (PDR; n = 7). Indications for vitrectomy for all patients included macular hole, epiretinal membrane, symptomatic vitreomacular traction, or persistent vitreous opacities. Age and gender distribution were generally comparable across groups, though the PDR group was notably younger (62.7 ± 3.6 years) than the CTRL (77.8 ± 12.9), DIA (71.4 ± 5.1), and NPDR (73.6 ± 5.7) groups. Male representation was highest in the CTRL group (83.3%) and lowest in the PDR group (28.6%). DM2 was present in all DIA patients, four of NPDR, and six of PDR patients. DM2 duration was longest in the PDR group (27 ± 9.5 years) and shortest in the DIA group (9.5 ± 0.7 years). Only the PDR group had received prior DR treatment, with five patients undergoing photocoagulation and three patients receiving anti-VEGF therapy 6–14 days prior to surgery. Controlled open-angle glaucoma was present in one patient in each of the CTRL and DIA groups.

Almost all patients were pseudophakic at the time of vitrectomy, and most diabetic participants had well-managed hypertension and dyslipidemia.

Vitrectomy indications and DR staging were assessed by consultant ophthalmologists (G.P., X.L.). NPDR was defined by the presence of microaneurysms, dot/blot hemorrhages, or exudates. PDR was defined by neovascularization (disc or elsewhere), preretinal or vitreous hemorrhage, preretinal fibrosis, or tractional retinal detachment.

Undiluted vitreous samples (0.2–1.2 mL) were collected during standard 25G PPV before infusion, aspirated from the mid-vitreous, transferred to sterile cryovials (Nunc; Thermo Fisher Scientific), snap-frozen in liquid nitrogen, and stored at –80°C.

Metabolomics and Statistical Analysis

Each 30 µL vitreous sample was mixed with 90 µL cold methanol (4°C), centrifuged at 21,000 RCF for 10 minutes at 4°C, and the supernatant was transferred to high-performance liquid chromatography vials. Blank samples used water in place of vitreous fluid. Samples were analyzed using global metabolomics in Full mass spectrometry mode, as described by Skogvold et al[30] employing identical instrumentation, solvents, and software.

Four pooled group samples (CTRL, DIA, NPDR, PDR) were prepared by combining equal volumes from all samples in each individual group. These were analyzed using data-dependent MS/MS (Top 5, stepped normalized collision energies: 20, 50, 80; dynamic exclusion: 10 s; resolution at m/z 200: 17,500 full width at half maximum; automatic gain control target: 5e5 ion counts; max injection time: 100 ms). A pooled quality control (PQC) sample was created by combining equal volumes from each group pool and injected every fifth run to monitor instrument stability under Full MS mode.

Raw data were processed in Compound Discoverer 3.3 using the "Untargeted Metabolomics with Statistics Detect Unknowns with ID using Online Databases and mzLogic" workflow template. Principal component analysis (PCA) plots and volcano plots were generated, and group differences were assessed by one-way ANOVA followed by Tukey post hoc tests.

Feature Identification

Metabolites with $p < 0.05$ and fold change >1.5 or <0.67 were selected for identification based on chromatographic features, signal quality, and relevance to known pathways. Feature identification was based on confidence levels adapted from Schymanski et al[31] ranging from Level 5 (lowest) to Level 1 (highest). Level 5 includes only m/z value and retention time. Level 4 involves a predicted molecular formula used for database searches (eg, ChemSpider, HMDB). Level 3 indicates putative candidates based on class-specific MS/MS fragments. Level 2 reflects a probable structure matched via MS/MS spectra with online databases (mzCloud, GNPS, MoNA, LipidMaps). Level 1 requires a full match in m/z, fragmentation pattern, and retention time with an in-house library of 491 metabolites analyzed under identical conditions.

Results

Metabolomic analysis was conducted on 23 samples (CTRL n = 6, DIA n = 5, NPDR n = 5 and PDR n = 7). One sample from the NPDR group was excluded from statistical analysis in positive ionization mode due to poor detection of signal. Eighty-two of the detected features were selected for identification. Seven of these were found to be significantly reduced in the disease groups compared to CTRL with potential for therapeutic supplementation (Table S1). These were successfully identified at a level of confidence 1 or 2.

Antioxidant, Anti-Inflammatory and Metabolic-Protective Metabolites

Ascorbate (Vitamin C) was significantly reduced in the NPDR group compared to both the CTRL ($p < 0.05$) and DIA groups ($p < 0.01$; Figure 1). This reduction correlated with the progression of DR, suggesting metabolic disturbances linked to oxidative stress.

Stachydrine (Proline Betaine) demonstrated complex behavior, with a significant reduction in the NPDR group compared to CTRL ($p < 0.001$) and DIA ($p < 0.01$), while in PDR, it showed an increase compared to NPDR ($p < 0.05$) (Figure 1). This suggests a dynamic response in metabolic regulation as DR progresses.

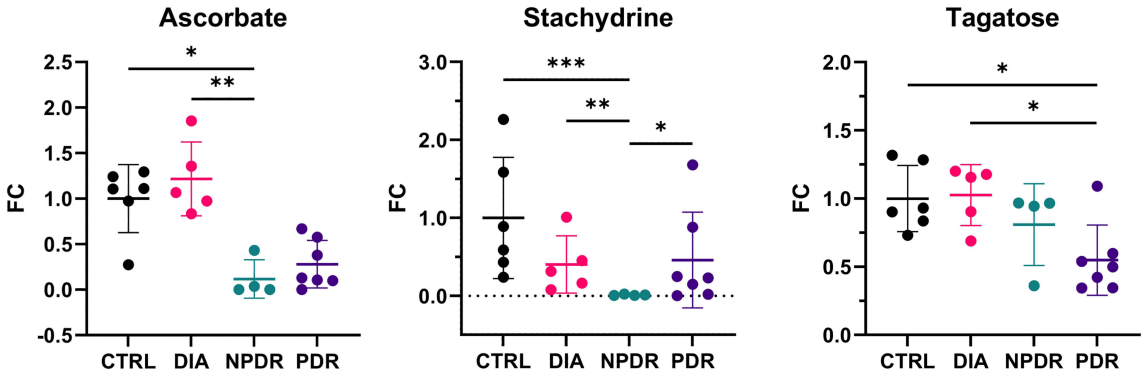

Figure 1 Scatter dot plots showing the trends in levels of antioxidant, anti-inflammatory and metabolic-protective molecules in the vitreous of the groups (mean ± SD). *p < 0.05; **p < 0.01; ***p < 0.001.

Abbreviations: FC, fold change vs control; CTRL, controls without diabetes or retinopathy; DIA, patients with diabetes and no retinopathy; NPDR, patients with non-proliferative diabetic retinopathy; PDR, patients with proliferative diabetic retinopathy.

Tagatose was significantly less abundant in the PDR group compared to both CTRL and DIA ($p < 0.05$), illustrating a larger metabolic alteration in the most advanced DR stage (Figure 1).

Metabolites for Neuronal Health

Choline levels were significantly reduced in the NPDR ($p < 0.05$) and PDR ($p < 0.01$) groups compared to CTRL, indicating possible dysregulation in phospholipid metabolism, which is essential for maintaining membrane integrity in retinal cells (Figure 2).

N-Acetylaspartic acid (NAA) showed a marked decrease in NPDR ($p < 0.05$) and PDR ($p < 0.01$) groups compared to CTRL (Figure 2), possibly reflecting neuronal damage and metabolic dysfunction in the retina.

Amino Acids

Taurine levels were significantly lower in NPDR compared to CTRL and DIA ($p < 0.05$), revealing a reduction of one of the most abundant retinal amino acids as retinopathy develops (Figure 3).

Serine showed a biphasic pattern in concentration, being significantly decreased in NPDR compared to CTRL ($p < 0.05$), but in higher levels in PDR compared to NPDR ($p < 0.05$; Figure 3). This might suggest a compensatory regulation of serine metabolism as DR advances, as it is important in cell membranes as part of phospholipids and sphingolipids.

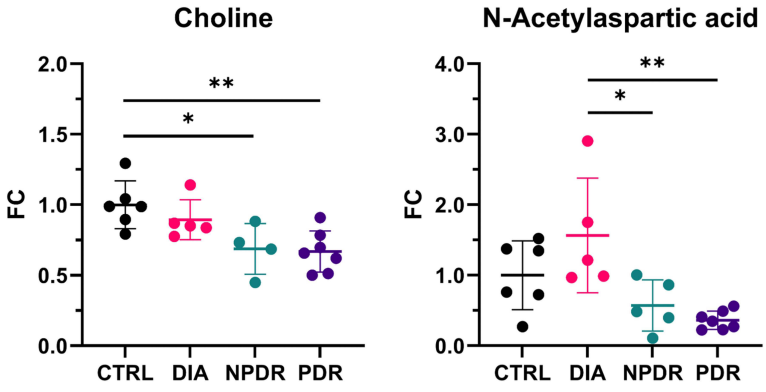

Figure 2 Scatter dot plots showing the trends in levels of neuronal health molecules in the vitreous of the groups (mean ± SD). Note that there are five dots in the NPDR group in the N-Acetylaspartic acid plot. This compound was detected in negative ionization mode where the fifth sample in the NPDR group had adequate signals. *p < 0.05; **p < 0.01.

Abbreviations: FC, fold change vs control; CTRL, controls without diabetes or retinopathy; DIA, patients with diabetes and no retinopathy; NPDR, patients with non-proliferative diabetic retinopathy; PDR, patients with proliferative diabetic retinopathy.

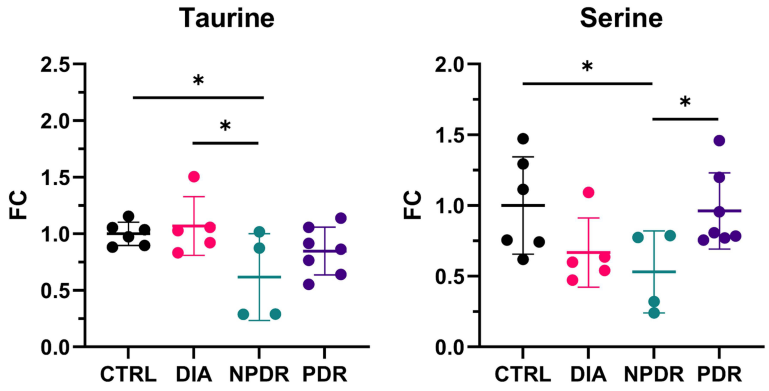

Figure 3 Scatter dot plots showing the trends in levels of amino acids in the vitreous of the groups (mean ± SD). *p < 0.05.
Abbreviations: FC, fold change vs control; CTRL, controls without diabetes or retinopathy; DIA, patients with diabetes and no retinopathy; NPDR, patients with non-proliferative diabetic retinopathy; PDR, patients with proliferative diabetic retinopathy.

Discussion

The results of this metabolomic analysis provide insights into the biochemical alterations occurring in the vitreous of patients with DR.

Ascorbate (vitamin C), a well-known antioxidant, was found to be significantly reduced in patients with NPDR compared to CTRL. Ascorbate is crucial in protecting the retina from oxidative stress by neutralizing reactive oxygen species.[32,33] The depletion of ascorbate in NPDR (Cohen's $d = 2.9$) suggests a compromised antioxidant defense system in the early stages of DR, which may contribute to retinal damage.[34,35] Previous research has shown that ascorbate is essential for various cellular functions, including collagen synthesis, vasodilation, and vascular reactivity, all of which are critical in maintaining retinal health under hyperglycemic conditions.[35,36] Supplementing ascorbate through the diet or as part of therapeutic strategies could, therefore, play a role in reducing oxidative damage and preventing the progression of DR. Although supplementation of ascorbate and other antioxidative vitamins have shown promising results in reducing glycemic profile in type 2 DM, further high-quality clinical studies are needed to evaluate the therapeutic and preventative effects on DR.[37–39]

Stachydrine (Proline Betaine) exhibited a complex trend, with levels significantly reduced in NPDR (Cohen's $d = 1.8$), but increased in PDR compared to NPDR (Cohen's $d = 1.0$). Stachydrine, commonly found in citrus fruits, has been shown to ameliorate endothelial cell dysfunction and insulin resistance in various models.[40,41] It has also been demonstrated to reduce inflammation and oxidative stress through pathways involving SIRT1 and AMPK, which are critical in DR pathogenesis.[41] Furthermore, studies have shown that stachydrine has strong anti-fibrotic and vasoprotective properties through multiple molecular mechanisms.[42] The relative increase in stachydrine levels in PDR compared to NPDR could reflect a compensatory metabolic response aimed at counteracting the progression of retinal damage. Given its protective properties, dietary supplementation of stachydrine through the consumption of citrus fruits or related compounds might offer therapeutic benefits in the earlier stages of DR to prevent disease progression.[43]

Tagatose, a hexose monosaccharide naturally present in dairy products and cocoa, was found at lower concentrations in the vitreous humor of patients with PDR compared to CTRL and DIA (Cohen's $d = 1.8$). This compound has been associated with several health benefits, including weight loss promotion, hypoglycemic effects, and symptom reduction in DM type 2.[44] Clinical studies have demonstrated that tagatose supplementation significantly lowers HbA1c levels in patients with DM compared to placebo, highlighting its potential as an anti-diabetic and anti-obesity agent.[45–48] Although no studies have specifically examined the therapeutic effects of tagatose supplementation in DR, its role in metabolic regulation suggests a promising preventive potential in the progression of retinopathy.

Choline levels were significantly reduced in both NPDR and PDR stages (Cohen's $d = 2.1$). Choline is a precursor for phosphatidylcholine, a critical component of cell membranes, particularly in neurons and endothelial cells.[49–52] Its deficiency may compromise membrane integrity, leading to increased retinal cell vulnerability and endothelial dysfunction in the microvasculature, which are hallmarks of DR.[53,54] Moreover, choline is the main constituent of acethylcholine,

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an important neurotransmitter and potent vasodilator secreted by retinal amacrine cells.[55] Studies have shown that impairment of the cholinergic activity of amacrine cells is associated with reduced blood flow in retinal vessels due to poor stimulation of the muscarinic receptors expressed on retinal pericytes and endothelial cells.[53,56–58] This finding aligns with existing literature linking choline to retinal neuronal membrane stability and lipid metabolism, as well as retinal vascular health.[59–62] In addition to retinopathy, choline deficiency has also been linked to complications such as nephropathy, cardiomyopathy and neuroinflammation in murine diabetic models, further emphasizing the potential vaso- and neuroprotective properties in DM.[52,63,64] Thus, dietary intake of choline-rich foods, such as eggs and fish, could potentially enhance retinal resilience by supporting phospholipid synthesis, membrane health and retinal blood flow, especially in diabetic patients at risk of DR progression.[53]

The observed decrease in NAA in both NPDR (Cohen's $d = 1.6$) and PDR (Cohen's $d = 2.1$) groups compared to CTRL suggests significant neuronal involvement as DR progresses. NAA is a marker of neuronal health and function, and its reduction could indicate early neuronal dysfunction or degeneration in the retina.[65–67] This aligns with previous studies suggesting that neurodegeneration plays a critical role in DR pathology.[14,68] While direct supplementation of NAA might not be feasible, diets rich in neuroprotective nutrients, such as polyunsaturated fatty acids and polyphenols, could help mitigate neuronal damage and provide a protective effect against retinal degeneration.[69–71]

Serine, a non-essential amino acid, plays a crucial role in cellular function as a key component of phospholipids and sphingolipids in cell membranes, making it essential for the development and function of the central nervous system.[72] In the retina, serine has been identified as a critical molecule for the phagocytic activity of the retinal pigment epithelium and neuronal communication within the inner retina, in addition to exhibiting antioxidant properties through serine-derived scavengers.[73] Preclinical studies have demonstrated that serine reduces insulin resistance, mitigates neuronal death, and lowers the incidence of DM type 1 in murine models, suggesting its potential therapeutic role in diabetes management.[74,75] Furthermore, inhibiting the conversion of naturally occurring L-serine to D-serine has been shown to attenuate retinal ganglion cell death and decrease the number of damaged acellular capillaries in animal models of DR.[76] Additionally, serine supplementation has been associated with beneficial effects on other microvascular complications of DM, including diabetic neuropathy and nephropathy.[77,78] In our study, serine concentrations exhibited a biphasic pattern across the stages of DR, with significantly lower levels observed in NPDR compared to both the CTRL and PDR groups (Cohen's $d = 1.5$). The subsequent increase in serine levels from NPDR to PDR may reflect a compensatory upregulation in response to neuronal degeneration and oxidative stress as DR progresses. These findings suggest that serine supplementation may hold therapeutic potential in diabetic patients for the prevention and management of diabetic retinopathy.

Taurine was significantly reduced in NPDR compared to CTRL and DIA groups (Cohen's $d = 1.4$). Taurine is one of the most abundant free amino acids in the retina, and plays a critical role in maintaining retinal homeostasis, including photoreceptor protection, antioxidant defense, and neurotransmission regulation.[79–82] Animal studies showed that taurine deficiency leads to severe retinal degeneration, and that dietary and intravitreal taurine administration protects against retinal ganglion cell loss, preserves photoreceptors, and restores retinal pigment epithelium function, as well as counteracts retinal neovascularization.[80,83] In diabetic models, taurine attenuates glutamate dysregulation in Müller cells, prevents glial cell alterations, and protects against oxidative stress-induced apoptosis in retinal and renal tissues.[84–86] Additionally, taurine reduces proteinuria and mitigates hyperglycemia-induced apoptosis in tubular cells, suggesting a role in the prevention of diabetic nephropathy.[84,87] Plasma taurine levels have been found to be significantly lower in patients with DR compared to diabetic patients without retinopathy, further implicating taurine depletion in DR progression.[83] Given its antioxidant, anti-inflammatory, and neuroprotective properties, taurine supplementation may represent a promising therapeutic strategy for preventing or mitigating DR progression in diabetic patients.

The identified deficiencies and trends in key metabolites suggest potential pathways for nutritional interventions to prevent, delay or mitigate the progression of DR (Figure 4a). Ascorbate levels are significantly reduced in NPDR compared to controls, indicating early oxidative stress. Therefore, ascorbate supplementation from the DIA stage onward may enhance antioxidant defenses and prevent oxidative damage. Similarly, choline levels decline significantly from DIA to NPDR and remain low in PDR, highlighting its potential role in maintaining cell membrane integrity and endothelial health. Continuous choline supplementation from DIA to PDR may provide protective benefits to retinal cells. The

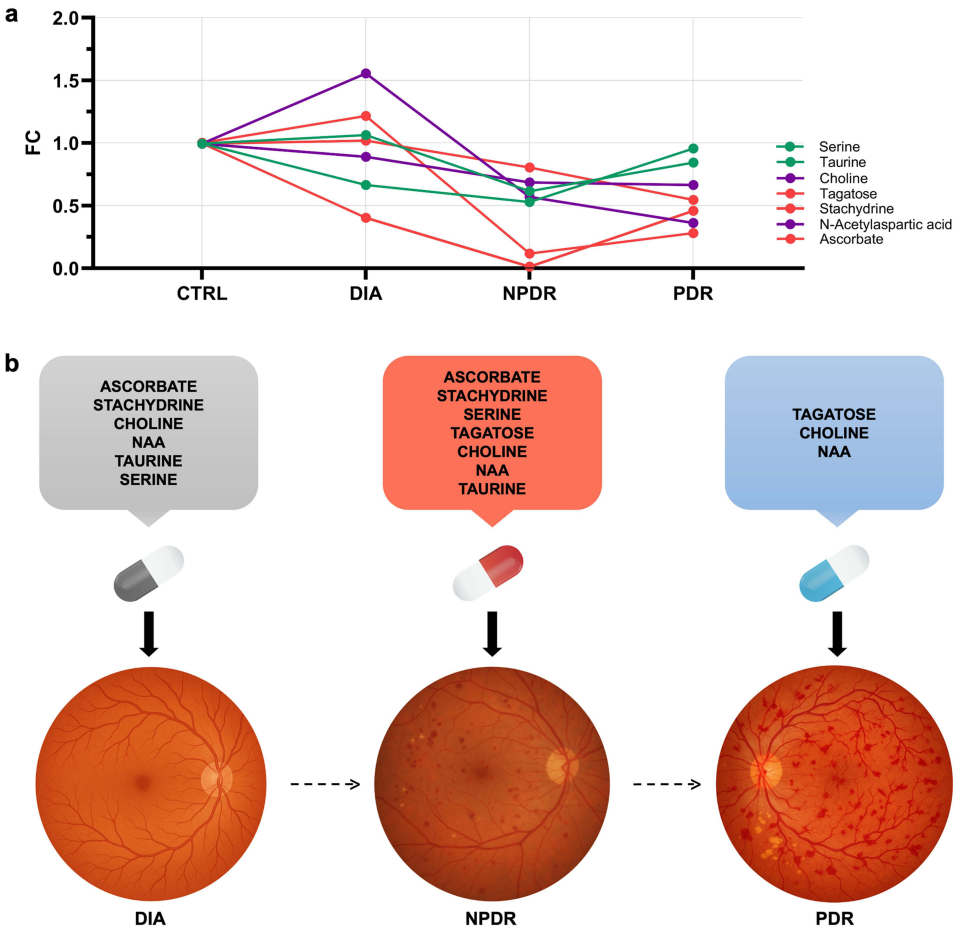

Figure 4 Key metabolites as potential therapeutic targets for nutritional supplementation in diabetic retinopathy stages. (**a**) Trends of detected differentially abundant metabolites (mean); anti-inflammatory/-oxidative (red), neuronal markers (purple), amino acids (green). (**b**) Potential supplemental strategy throughout the diabetic retinopathy stages for the prevention and treatment of disease progression. Animated fundus images generated by ChatGPT (OpenAI, 2025).
Abbreviations: FC, fold change vs control; CTRL, controls without diabetes or retinopathy; DIA, patients with diabetes and no retinopathy; NPDR, patients with non-proliferative diabetic retinopathy; PDR, patients with proliferative diabetic retinopathy; NAA, N-Acetylaspartic acid.

observed reduction in NAA in both NPDR and PDR suggests ongoing neuronal damage. While direct supplementation of NAA may not be feasible, a diet rich in neuroprotective compounds, such as the omega-3 fatty acids like docohexaenoic acid, eicosapentanoic acid, polyphenols and flavonoids, could support retinal neuronal health. Serine, like stachydrine, may also play a crucial role in neuronal protection, particularly in the later stages of DR, where its seemingly compensatory upregulation suggests an endogenous response to ongoing retinal damage. Supplementing serine from DIA to PDR could mitigate neurodegeneration and support neuronal survival and retinal function. Moreover, taurine supplementation should be initiated at the DIA stage for the prevention of retinopathy development, as its antioxidant, neuroprotective, and anti-inflammatory effects have been shown to reduce retinal cell apoptosis, preserve synaptic integrity, and counteract oxidative stress. Tagatose supplementation from NPDR may also be beneficial, given the significant metabolic alterations occurring in PDR compared to CTRL and DIA. Its hypoglycemic and metabolic-regulating properties may help mitigate hyperglycemia-induced retinal damage.

Collectively, these findings emphasize the potential of targeted nutritional strategies in modifying the metabolic profile of DR and slowing its progression (Figure 4b). By addressing key metabolic deficiencies at different disease stages, these interventions may offer a promising approach for DR prevention and management.

This study offers insights into progressive vitreous metabolic deficiencies in DR and potential nutritional therapeutic targets, but several limitations should be noted. As in previous research, CTRL patients underwent vitrectomy for macular holes or epiretinal membranes, which may not fully represent healthy ocular metabolism.[19,20,22,24] However,

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vitrectomy in truly healthy eyes is not ethically feasible, making these conditions a practical proxy. Similarly, DIA patients had surgery for macular holes or vitreomacular traction, as operating on asymptomatic diabetic eyes is not viable. Age and diabetes duration varied across groups, both of which can influence the metabolome directly or via comorbidities and treatments.[88,89] These factors may partially explain the observed metabolic differences. Future studies should stratify or adjust for these variables. The relatively small sample size, especially in the DIA and NPDR groups, limits generalizability and precludes subgroup analyses of anti-VEGF treatment. As a result, some statistically significant differences observed may be attributable to random variation; however, effect size estimates (Cohen's *d*) were calculated to emphasize the clinical relevance of the findings, with all effect size estimate values indicating large significance (Cohen's $d > 0.8$). Larger cohorts are needed to evaluate treatment-related metabolic effects and validate findings. Finally, only the vitreous metabolome was analyzed, based on its proximity to retinal processes. Future studies should include blood metabolomics to better understand systemic contributions to DR progression.

Conclusion

The metabolic disturbances observed in this study underscore the potential of dietary supplementation as an adjunctive strategy for managing DR. The progressive dysregulations in ascorbate, choline, tagatose, NAA, stachydrine, taurine and serine suggest that targeted nutritional interventions could help restore metabolic function and mitigate retinal damage. Future research should focus on clinical trials evaluating the efficacy of dietary interventions aimed at replenishing these key metabolites to prevent the progression of DR.

Abbreviations

AMPK, 5′ AMP-activated protein kinase; CTRL, Control; DIA, Diabetes without retinopathy; DM, Diabetes mellitus; DR, Diabetic retinopathy; FC, Fold change; MS, Mass spectrometry; NPDR, Non-proliferative diabetic retinopathy; NAA, N-Acetylaspartic acid; PCA, Principal component analysis; PDR, Proliferative diabetic retinopathy; PPV, Pars plana vitrectomy; SIRT1, Sirtuin 1.

Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate

Provided in the Patients and Methods section.

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Disclosure

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