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Vitamin A aldehyde-taurine adducts function in photoreceptor cells

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ABSTRACT

To facilitate the movement of retinoids through the visual cycle and to limit nonspecific chemical reaction, multiple mechanisms are utilized to handle these molecules when not contained within the binding pocket of opsin. Vitamin A aldehyde is sequestered by reversible Schiff base formation with phosphatidylethanolamine (PE) and subsequently undergoes NADPH-dependent reduction. Otherwise inefficient handling of retinaldehyde can lead to the formation of fluorescent di-retinal compounds within the outer segments of photoreceptor cells. These bisretinoid fluorophores initiate photooxidative processes having adverse consequences for retina. Various carrier proteins confer water solubility and maintain the 11-*cis*-retinoid configuration. Mechanisms for sequestration of retinoid include the formation of a reversible Schiff base between retinaldehyde and taurine (A1-taurine, A1T), the most abundant amino acid in photoreceptor cells. Here we have undertaken to examine the effects of taurine depletion using the transport inhibitors guanidinoethyl sulfonate (GES) and β-alanine. Oral treatment of BALB/cJ mice with β-alanine reduced ocular A1T and the mice exhibited significantly lower scotopic and photopic a-wave amplitudes. As a secondary effect of retinal degeneration, A1T was not detected and taurine was significantly reduced in mice carrying a P23H opsin mutation. The thinning of ONL that is indicative of reduced photoreceptor cell viability in albino *Abca4*$^{-/-}$ mice was more pronounced in β-alanine treated mice. Treatment of agouti and albino *Abca4*$^{-/-}$ mice with β-alanine and GES was associated with reduced bisretinoid measured chromatographically. Consistent with a reduction in carbonyl scavenging activity by taurine, methylglyoxal-adducts were also increased in the presence of β-alanine. Taken together these findings support the postulate that A1T serves as a reservoir of vitamin A aldehyde, with diminished A1T explaining reduced photoreceptor light-sensitivity, accentuated ONL thinning in *Abca4*$^{-/-}$ mice and attenuated bisretinoid formation.

1. Introduction

Taurine (2-aminoethanesulfonic acid) is an endogenous sulfur-containing β-amino acid that is not incorporated into protein but is the most abundant constituent of the amino acid pool of photoreceptor cells [1]. Although taurine can be synthesized endogenously from cysteine, it is primarily acquired through the diet [1,2]. The structure of taurine does not include the carboxyl group typical of amino acids; instead, it bears a sulfonate group. Retinal pigment epithelial (RPE) cells take up considerable amounts of taurine; only the uptake of proline is higher [3]. Taurine enters RPE cells by means of a high-affinity Na^+ and Cl^- dependent taurine transporter (TauT; SLC6a6) [4–6]. High concentrations of free taurine accumulate in neural retina in association with photoreceptor cells [1]. Structural analogs of taurine, including the synthetic compound guanidinoethyl sulfonate (GES) [7] and natural occurring β-amino acids such as β-alanine [8] are competitive inhibitors of the taurine transporter [6,9]. Both glutathione (GSH) and taurine rely on cysteine for synthesis. It is perhaps for this reason that taurine supplementation may increase reduced GSH levels [10]. Aging is reported to be associated with decreased taurine in plasma and tissues [11].

Taurine deficiency negatively impacts the retina. Recently, there have been two reports of unrelated families with affected siblings carrying homozygous disease-causing missense variants (p.G399V; p.A78E) in the solute carrier (SLC) 6a6 gene [5,12] that encodes TauT [2]. These hypomorphic alleles conferred profound reductions in taurine transport [12], meager plasma taurine levels, cardiomyopathy and early-onset

Abbreviations: ERG, electroretinography; HPLC, high performance liquid chromatography; MG, methylglyoxal; ONL, outer nuclear layer; qAF, quantitative fundus autofluorescence; RPE, retinal pigment epithelial; UPLC, ultra performance liquid chromatography.

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retinal degeneration. The latter was evidenced by thinning of photoreceptor-attributable layers in spectral domain optical coherence tomography (SD-OCT) scans and severe reductions in electroretinograms [12]. Oral taurine supplementation (100 mg/kg/day, 2 years) enabled normal blood taurine levels, correction of the cardiomyopathy and non-progression of retinopathy in a 6-year-old [12]. Interest in taurine also originates from reports that the bilateral concentric visual field loss that is a side-effect of the anti-epileptic drug vigabatrin (GABA-transaminase inhibitor) is due to taurine depletion [2,13,14]. The photoreceptor cell degeneration occurring as a side-effect of vigabatrin is intensified by light exposure and albino mice are predisposed [15].

Taurine carries a primary amine that in retina reversibly binds to retinaldehyde thereby forming a small molecule, taurine-retinaldehyde Schiff base (A1T) [16,17]. A1T is an amphiphilic molecule carrying a negatively charged sulfonated group together with a hydrophobic retinoid moiety and may serve to chaperone retinaldehyde, a molecule that is otherwise poorly soluble in aqueous media. We recently showed that A1T is present at higher levels in neural retina as opposed to RPE and is more abundant under conditions in which 11-*cis*-retinaldehyde is more abundant [16,17]. These conditions included mice carrying the Rpe65-Leu450 versus Rpe65-450Met variant, the presence versus absence of ocular pigmentation, dark-adaptation versus light-adaptation. A1T was also substantially decreased in mouse models in which the synthesis of 11-*cis*-retinal is compromised, specifically *Rpe65*$^{-/-}$ and *RLBP1*/CRALBP$^{-/-}$ mice. These findings indicated that, as an amphiphilic molecule A1T may represent a mechanism for escorting retinaldehyde in an aqueous milieu and may signify a pool of mobilizable 11-*cis*-retinal [17].

The ability of taurine to sequester retinaldehyde is of additional interest. Although 11-*cis*-retinaldehyde is the visual chromophore that absorbs photons of light to begin the process of phototransduction, retinaldehyde is also highly reactive and thus chaperoning during transit through the visual cycle and chemical reduction of the aldehyde are important protective processes [18–20]. The carrier proteins that serve to chaperone retinoid include cellular retinaldehyde-binding protein (CRALBP), interphotoreceptor retinoid-binding protein (IRBP) and cellular retinoid binding protein (CRBP). Within photoreceptor cells 11-*cis*-retinaldehyde also binds to opsin and to phosphatidylethanolamine via Schiff base linkages. One consequence of inefficient handling of retinaldehyde is the potential for reaction of two of the aldehydes with phosphatidylethanolamine in the photoreceptor outer segment membrane, the outcome being the formation of toxic bisretinoid compounds. Evidence of adverse effects of bisretinoid has been provided by studies demonstrating that bisretinoids undergo photooxidation and subsequently degrade into small carbonyl compounds including glyoxal and methylglyoxal [21–24]. These aldehyde-bearing species are known to be reactive; they randomly attack amine moieties of biomolecules forming advanced glycation endproducts (AGEs), that are deleterious to cells.

Here we have investigated A1T, retinoid and bisretinoid levels under conditions of taurine depletion and taurine supplementation and we have measured A1T in mouse models of retinitis pigmentosa (RP) and recessive Stargardt disease (*Abca4*$^{-/-}$ mice). Since as a free amino acid taurine is present at high concentration in retina, we have also explored its ability to quench the activity of dicarbonyl aldehydes released by bisretinoid photooxidation.

2. Materials and methods

2.1. Mouse models

BALB/cJ mice and P23H knock-in mice (*Rho*$^{P23H/P23H}$) homozygous mice [25] were purchased from the Jackson Laboratory (Bar Harbor, ME). The albino *Rho*$^{P23H/+}$ mice were custom generated at The Jackson Laboratory by repeated crossing homozygous black P23H mice (JAX stock# 017628) to albino B6 mice (JAX stock# 000058) for over 12 generations and the genetic background was confirmed to be over 99% of that of albino B6. Both albino homozygous mice and their littermate controls were maintained in house. Albino *Abca4* null mutant mice (*Abca4*$^{-/-}$); albino *Abca4*$^{+/+}$ mice; and agouti *Abca4* null mutant mice (*Abca4*$^{-/-}$) (The Jackson Laboratory) all of which were homozygous for Rpe65-Leu450, were bred in the laboratory and housed under 12-h on-off cyclic lighting with in-cage illuminance of 30–80 lux. To achieve dark-adapted conditions, mice were placed in darkness for 18–20 h. Genotyping confirmed that the mutant mice do not carry the rd1, 2, 3, 6, 7, 8 and 10 mutations [26]. Mouse lines studied are provided in Table 1. The research was approved by the Institutional Animal Care and Use Committee (IACUC) and was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

2.2. Taurine, β-alanine and GES treatment

Mice received oral taurine (Millipore-Sigma, St. Louis, MO; pharmagrade) delivered as 2.5% (w/v) in drinking water for 2 and 6 months. Guanidinoethyl sulfonate (GES) was synthesized by Toronto Research Chemicals (Toronto, Ontario; pharmagrade) and delivered in drinking water (1%) for 2 months. β-alanine (NutriVitaShop, Lake Forest, CA; pharmagrade) was delivered to mice in drinking water (2%) for 1–2 months.

2.3. In vitro cellular experiments

Human adult RPE (ARPE-19, American Type Culture Collection, Manassas, VA) were treated with GES and β-alanine where indicated. To test for direct effects of taurine on cultured RPE, ARPE-19 cells deficient in endogenous lipofuscin [27] were grown to confluence as described [28]. The cells accumulated A2E with and without β–alanine for 14 days. After culturing for an additional 3 days in media without A2E, the cells were incubated with taurine (1 mM) for 24 h and without further incubation the cells were exposed to 430-nm light (30 min). After 18 h, the MTT (4, 5-di-methylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay (Roche Diagnostics, Indianapolis, IN) was performed to evaluate cell viability.

2.4. Synthesis

A2E, A2-GPE (A2-glycerophosphoethanolamine), A2-DHP-PE (A2-dihydropyridine-phosphatidylethanolamine), atRALdi-PE (all-*trans*-retinal dimer-phosphatidylethanolamine) and atRALdi were synthesized as previously described with HPLC purification [23,27,29,30]. Retinoids (9-*cis*-retinal, *13*-cis-retinal and all-*trans*-retinal) were purchased from Sigma-Aldrich; 11-*cis*-retinal was a gift from the National Eye Institute provided through the laboratory of Dr. Rosalie K. Crouch. A1-taurine isomers were synthesized as published [31]. A1-taurine isomers were purified in their protonated forms by HPLC with a reverse-phase Atlantis Prep dC18 column (10 × 250 mm, 10 μm; Waters) and gradient of acetonitrile and water with 0.1% TFA [17].

2.5. Quantitative UPLC analysis of retinoids

Frozen mouse eyecups (1 eye/sample) were homogenized and derivatized with *O*-ethylhydroxylamine (100 mM) (method 1) or with *O*-ethylhydroxylamine (100 mM) and acetonitrile (method 2) on ice under dim red light. Retinoids were extracted with hexane and resuspended in acetonitrile for UPLC analysis [32] using a Waters Acquity UPLC-PDA system (Milford, MA) with a CSH C18 column as described [33]. Molar quantities per eye were calculated based on standard solutions with concentrations determined spectrophotometrically. Peak areas were calculated using Waters Empower Software and results were analyzed in Excel (Microsoft, Redmond, WA).

Table 1
Mouse lines included in the study.

Genotype	Treatment in drinking water (w/v)	Age (months)	Light-condition	Measurement	Corresponding figure
BALB/cJ	β-alanine 2%	1–3	cyclic light	taurine in plasma	Fig. 1B
				taurine in eyes	Fig. 1C
				A1T	Fig. 1D
		1–3	dark-adaptation	retinoids	Fig. 2A and B
		2–3	light-adaptation		Fig. 2C and D
		1–3	cyclic light	ERG	Fig. 3
		2–3	2 h post-bleach	retinoids	Fig. 4A and B
		1–3	6 h post-bleach		Fig. 4C and D
Albino *Rho*$^{+/+}$ *Rho*$^{P23H/+}$	No treatment	2	cyclic light	taurine in eyes	Fig. 5A
		1	dark-adaptation	retinoids	Fig. 5B
		2	cyclic light	A1T	Fig. 5C
albino *Abca4*$^{+/+}$ *Abca4*$^{-/-}$		4		taurine in eyes	Fig. 5D
		3	dark-adaptation	retinoids	Fig. 5E
		3	cyclic light	A1T	Fig. 5F
albino *Abca4*$^{-/-}$	β-alanine 2%	1–3	cyclic light	bisretinoids	Fig. 6A–C
agouti *Abca4*$^{-/-}$	GES 1%				Fig. 6D and E
	β-alanine 2%				Fig. 6F and G
albino *Abca4*$^{-/-}$	taurine 2.5%	1–3		bisretinoids	Fig. 7
	β-alanine 2%	6–8		ONL	Fig. 8

ERG, electroretinography; GES, guanidinoethyl sulfonate; ONL, outer nuclear layer.

2.6. Quantitation of bisretinoids

Mouse eyecups were homogenized, extracted with either A or B (A: homogenized in Dulbecco's phosphate-buffered saline (DPBS) and extracted in chloroform/methanol, 1:1; B: homogenized and extracted in chloroform/methanol, 1:1) and the solvent was evaporated as previously described [34]. The extract was re-dissolved in chloroform/methanol by HPLC and in ethanol by UPLC for bisretinoid measurement (Waters, Milford, MA) [35]. One to eight eyes were combined for bisretinoid measurement, each measurement was expressed as picomoles/eye, and from multiple measurements, mean values were determined.

2.7. HPLC quantitation of taurine

RPE cells and whole murine eyes or isolated neural retina and isolated RPE/choroid were homogenized in DPBS and extracted in chloroform and methanol (1:1) to exclude the non-polar fraction. The DPBS fraction was derivatized with NBD-F solution (1 mmol/L) as described [36]. An Alliance system (Waters, Milford, MA) equipped with a 2475 Multi λ fluorescence detector (Ex: 470 nm, Em: 530 nm) was used for HPLC analysis as previously described [17]. Molar quantity of taurine was determined using standard curves.

2.8. UPLC quantitation of A1T isomers

Whole mouse eyes (3–4 eyes/sample) were homogenized in acidified MeOH (0.1% TFA) and extracted in chloroform/DPBS (2: 1.3) (on ice and under dim red light). Acidified methanol was used to protonate the primary amine of taurine thus rendering it incapable of nucleophilic attack on the aldehyde moiety of retinaldehyde. The addition of PBS in the biphasic system with chloroform prevented unwanted formation of A1T during extraction [17]. The sample was then centrifuged, filtered, and extracted twice by addition of chloroform. The combined chloroform fraction was dried and then redissolved in MeOH and measured by a Waters Acquity UPLC system (Milford, MA) with an XBridge BEH C18 column. A1-taurine isomers 11-*cis*, 13-*cis*, 9-*cis*, all-*trans*-retinal were separated with water and acetonitrile, both with 0.1% formic acid as published [17]. Molar quantities per eye were calculated by comparison with each of the synthesized A1T isomers. To calculate total A1T, the isomers were quantified separately and then summed [17]. A1-β-alanine was added as an internal standard to compensate for Schiff base hydrolysis during the extraction. In desiccated mouse eyes extracted in dehydrated methanol that favors reactions between endogenous taurine and β-alanine with free retinaldehyde we confirmed that most of the Schiff base was A1T (99%) and a small portion of Schiff base was A1-β-alanine (1%). This is consistent with previous findings [37].

2.9. Measurement of outer nuclear layer (ONL) thickness

Mouse eyes were marked and fixed as described [38]. Sagittal paraffin serial sections of mouse retina were prepared and stained with hematoxylin and eosin. The section most centrally positioned in the optic nerve head (ONH) was selected and imaged with the 40 × objective using Leica SCN 400 system (Leica Microsystems; Leica Application suite; Welzlar, Germany). ONL thickness was then measured at 200 μm intervals superior and inferior to the edge of the ONH along the vertical meridian with image processing software Fiji after a custom ruler was place with Photoshop CS5. ONL width in pixels was converted to microns (1 pixel: 1.26 μm). For groups of mice at the indicated ages, mean ONL thickness of 3 sections at each position along the vertical plotted as a function of eccentricity from the ONH [39].

2.10. Electroretinography (ERG)

ERG recordings were obtained with a Celeris rodent ERG system (Diagnosys, Lowell, MA). Scotopic and photopic responses were recorded under incremental stimuli. To measure recovery of visual sensitivity, the eyes were exposed to 1000 cd s/m^2 white light for 5 min to bleach rhodopsin (90%) and ERG responses to 1 cd s/m^2 white light were recorded before and after bleaching at regular intervals.

2.11. Quantitative fundus autofluorescence (qAF)

Short-wavelength (SW) fundus autofluorescence (488 nm excitation) images were acquired non-invasively from mice as described [40].

2.12. Detection of methylglyoxal-(MG) adducts by enzyme-linked immunosorbent assay (ELISA)

Dissected neural retina and RPE (8 eyes per sample) were placed in 1X lysis buffer (Cell Signaling, Danvers, MA) with 1% (v/v) protease inhibitors (protease inhibitor cocktail, Millipore-Sigma, St. Louis, MO) and were sonicated on ice. After centrifuging the lysates, the MG-H1 (methylglyoxal-hydroimidazolone) protein adducts were quantified by competitive indirect enzyme-linked immunosorbent assay (ELISA) using an HRP conjugated-secondary antibody (OxiSelect, Cell Biolabs, San Diego, CA). MG content was determined by comparison with an MG-BSA

standard curve, a four-parameter fit algorithm [41]. To ensure antibody specificity for MG-H1, an adduct of MG with an arginine residue and a reaction mixture of MG with taurine were screened.

2.13. Statistical analysis

Statistical analysis was carried out using GraphPad *Prism*, version 8 (GraphPad Software, Inc. La Jolla, CA*) and* $P < 0.05$ was considered significant.

3. Results

3.1. Depletion of taurine by GES- and β-alanine-treatment in ARPE-19 cells and in mice

The taurine transporter (TauT) a sodium- and chloride-dependent protein encoded by the SLC6A6 gene and expressed by RPE is competitively inhibited by analogs of taurine such as guanidinoethyl sulfonate (GES) and β-alanine [7,42]. Since expression of TauT by ARPE-19 cells has been demonstrated previously by immunocytochemical staining [43], we confirmed the inhibitory behaviors of β-alanine and GES by HPLC quantification of taurine in ARPE-19 cells that were grown in media (with 10% fetal bovine serum) containing 0.4 microM taurine and treated with 0.5 and 2 mM of β-alanine and GES. In these GES- and β-alanine-treated cells, taurine concentration, measured after derivatization of amine groups by NBD-F, was inhibited by 96% and 97%, respectively ($P < 0.01$, 1-way ANOVA and Tukey's multiple comparison test) (Fig. 1A). We also quantified taurine in plasma and in isolated neural retina and RPE of mice. In cyclic reared albino BALB/cJ mice receiving β–alanine in drinking water from age 1–3 months, HPLC analysis revealed 19% lower levels of taurine in plasma ($P < 0.05$, unpaired two-tailed *t*-test) (Figs. 1B) and 14% decreased taurine in isolated neural retina ($P < 0.001$, two-way ANOVA and Bonferroni's multiple comparison test) versus untreated-controls (Fig. 1C). Conversely, in isolated RPE there was no appreciable difference in taurine levels between control and treated mice (Fig. 1C). Since β-alanine and GES produced similar levels of inhibition, and β-alanine was more readily available, we continued the work using β-alanine.

3.2. A1T levels in β-alanine-treated WT mice

A1T was also measured in eyes of BALB/cJ mice raised under cyclic light and treated with β-alanine from 1 to 3 months of age. Ocular A1T was reduced by 35% ($P < 0.01$; unpaired two-tailed *t*-test) in the β-alanine-treated versus control mice (Fig. 1D).

3.3. Effect of β-alanine on retinoid levels

We have previously shown that levels of A1T are higher under conditions in which 11-*cis*-retinal is more abundant. For instance, A1T (sum of 9-*cis*, 11-*cis*, 13-*cis* and all-*trans* isomers) is significantly greater in dark-adapted albino mice (C57BL/6J^{c2j} and BALB/cJ mice) relative to the same strains of cyclic light-adapted mice [17]. A1T is also higher in black C57BL/6J mice as compared to albino C57BL/6J^{c2j} mice. In the current studies BALB/cJ mice were treated with β-alanine from 1 to 3 months of age and then dark-adapted. In the treated mice there were no differences in retinoid (atROL, atRE, atRAL, 11cisRAL, 11cisRE) levels as compared to untreated mice. In the β-alanine-treated mice, there was, however, a decrease in 11cisROL (28% decrease) ($P < 0.05$, two-way ANOVA, and Sidak's multiple comparison test) (Fig. 2A and B). Similarly in cyclic-light reared BALB/cJ mice, there was no difference in retinoid levels in mice receiving oral β-alanine for 1 month compared with the untreated mice (Fig. 2C and D). We note that the estimated total retinoid concentration in dark vs light-adapted eyes is 500.9 ± 66.5 pmoles/eye and 700.6 ± 90.5 pmoles/eye, respectively (extraction method 1).

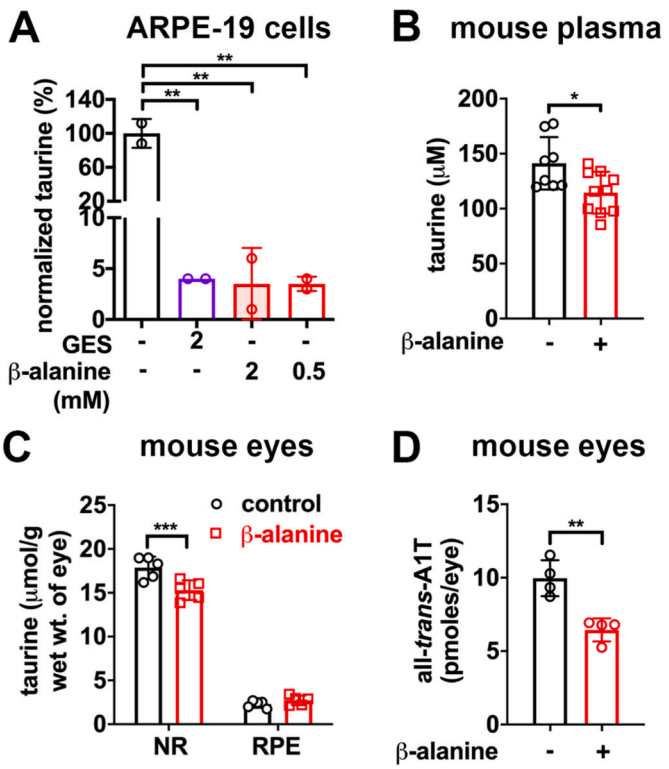

Fig. 1. Quantitation of taurine and A1T. Taurine and all-*trans*-A1T were measured by HPLC and UPLC, respectively. (A) Taurine in ARPE-19 cells was depleted by treating with the taurine transport inhibitors guanidinoethyl sulfonate (GES; 2 mM) and β-alanine (0.5 and 2 mM). (B–D) Cyclic light reared BALB/cJ mice treated with β-alanine (2% in drinking water; age 1–3 months). Taurine was measured in plasma (B) and in isolated neural retina (NR) and retinal pigment epithelium (RPE) (C). (D) A1T in β-alanine-treated mice was measured in whole eyes. Each plotted value is based on one sample (A) one mouse (B), one eye (C) and 4 eyes (D). Means ± SD based on 2 (A) 8–10 (B) 4–5 (C) 4 (D) replicates. *P* values were determined by one way ANOVA and Tukey's multiple comparison test (A), unpaired *t*-test (B, D) and two-way ANOVA with Sidak's multiple comparison test (C); $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

3.4. Electroretinographic recording in taurine-deficient mice

To assess retinal function in terms of both rod and cone activity, we also recorded scotopic and photopic electroretinographic responses in 3-month-old BALB/cJ mice treated with β-alanine for 2 months to reduce taurine uptake and we compared the data to untreated mice. The cornea-negative a-wave originates from photoreceptor cells while the b-wave reflects responses from bipolar cells [44]. As shown previously in taurine-deficient mice [45], β-alanine-treated mice exhibited significantly lower scotopic and photopic a-wave amplitudes ($P < 0.05$, 1-way ANOVA and Tukey's multiple comparison test) (Fig. 3A), and significantly increased b-wave implicit times ($P < 0.01$, 1-way ANOVA and Tukey's multiple comparison test) relative to control mice (Supplementary Fig. S1). B-wave amplitudes were unchanged (Fig. 3B). The changes in a-wave amplitude without corresponding changes in b-wave amplitudes likely indicates that the photoreceptor cell responses were adequate to elicit responses in bipolar cells; transmission of signal from photoreceptors to second order neurons is non-linear [46].

3.5. Effect of β-alanine on the recovery of retinoid levels 2, 6 h postbleach in dark-adapted BALB/cJ mice

Retinoids were analyzed in β-alanine-treated mice and controls when mice were dark-adapted overnight, subjected to photobleaching and allowed to recover for 2 and 6 h in the dark (Fig. 4). After 2 and 6 h of

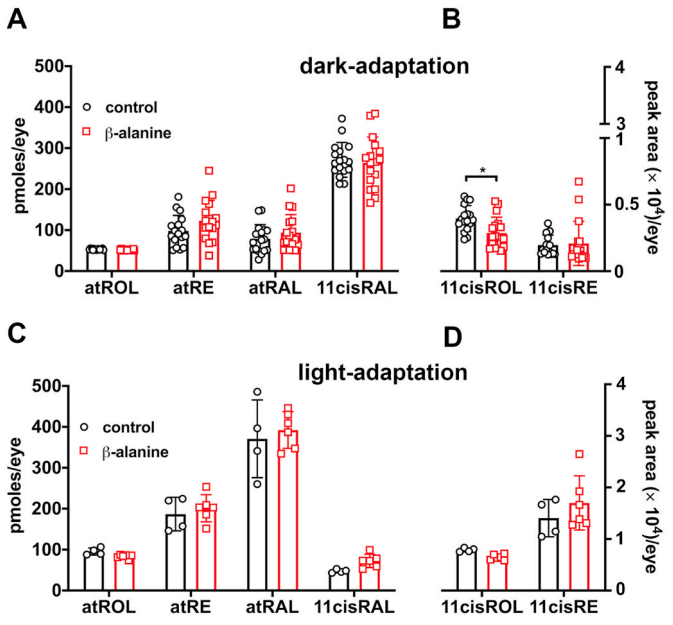

Fig. 2. UPLC quantitation of retinoids in *β*-alanine (2% in drinking water) treated and untreated (control) albino BALB/cJ mice (age 3 months). (A–D) Dark-adapted mice treated with *β*-alanine from age 1–3 months. (C, D) Cyclic light-adapted mice treated with *β*-alanine from age 2–3 months. On the left axis, all-*trans*-retinol (atROL), all-*trans*-retinyl palmitate (atRE), all-*trans*-retinal (atRAL), 11-*cis*-retinal (11cisRAL) are presented as picomoles per eye. On the right axis, 11-*cis*-retinol (11cisROL) and 11-*cis*-retinyl ester (11cisRE) are presented as peak area/eye. Extraction method 1. Individual values are based on one eye; $n = 4$–17, mean ± SD. *P* values were determined by two-way ANOVA and Sidak's multiple comparison test; $^{*}P < 0.05$.

recovery, there were no significant differences in retinoid levels in BALB/cJ mice treated with *β*-alanine for 1 and 2 months ($P > 0.05$) (Fig. 4).

3.6. A1T, retinoid and taurine levels in a mouse model of retinitis pigmentosa

A point mutation of proline to histidine at position 23 of opsin (P23H) is responsible for a dominant form of retinitis pigmentosa (RP) [47]. Partial misfolding of rhodopsin in P23H mutant mice results in abnormal 11-*cis*-retinal binding to the opsin protein [48]. In pigmented $Rho^{P23H/+}$ knock-in mice at age 63 days the ONL consists of 6–7 rows of photoreceptor cell nuclei as compared to 9–10 rows in wild-type mice [25]. We found that the concentration of taurine in the whole eyes (including neural retina) of albino albino $Rho^{P23H/+}$ mice was decreased by 31.7% when compared to $Rho^{+/+}$ control mice (Fig. 5A). Since numerous findings indicate that in retina taurine functioning primarily relates to photoreceptor cells [2,12,15,49–52], we tested for a secondary effect of reduced retinoid and photoreceptor cell degeneration on A1T levels. Accordingly, in albino $Rho^{P23H/+}$ knock-in mice at age 1 month we observed that 11-*cis*-retinal was reduced by 85% and atRAL was 69% lower (Fig. 5B). All-*trans*-retinyl ester, all-*trans*-retinal and 11-*cis*-retinal were also significantly reduced in the mutant mice ($P < 0.0001$, 2-way ANOVA and Sidak's multiple comparison test) (Fig. 5B). A1T was measured and compared in albino cyclic light-housed mice. The latter taurine-retinaldehyde Schiff base [17], was not detected in ocular

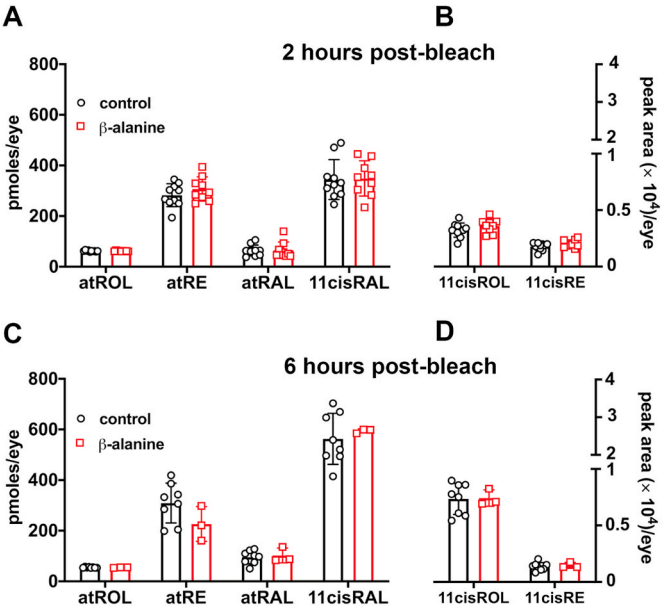

Fig. 4. Photobleaching and recovery. UPLC quantitation of retinoids in BALB/cJ mice treated with *β*-alanine for 1 (A, B) and 2 (C, D) months to deplete taurine. Mice were exposed to bleaching light and then allowed to recover for 2 (A, B) and 6 (C, D) hours in the dark. On the left axis all-*trans*-retinol (atROL), all-*trans*-retinyl palmitate (atRE), all-*trans*-retinal (atRAL), 11-*cis*-retinal (11cisRAL) are presented as picomoles per eye. On the right axis 11-*cis*-retinol (11cisROL) and 11-*cis*-retinyl ester (11cisRE) are presented as peak area/eye. Extraction method 2. Individual values are based on one eye; $n = 3$–10, mean ± SD. *P* values were determined by two-way ANOVA and Sidak's multiple comparison test.

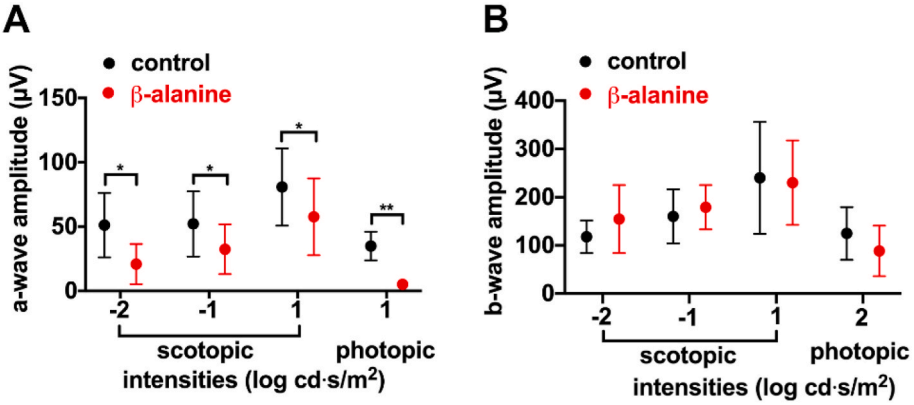

Fig. 3. Electroretinographic analysis of albino BALB/cJ mice treated orally with *β*-alanine from age 1–3 months. (A) a-wave amplitudes. (B) b-wave amplitudes. Amplitudes are plotted as a function of light-stimulus intensity for scotopic and photopic ERG recordings. Mean ± SD, $n = 6$. *P* values were determined by one-way ANOVA and Tukey's multiple comparison test. $^{*}P < 0.05$; $^{**}P < 0.01$.

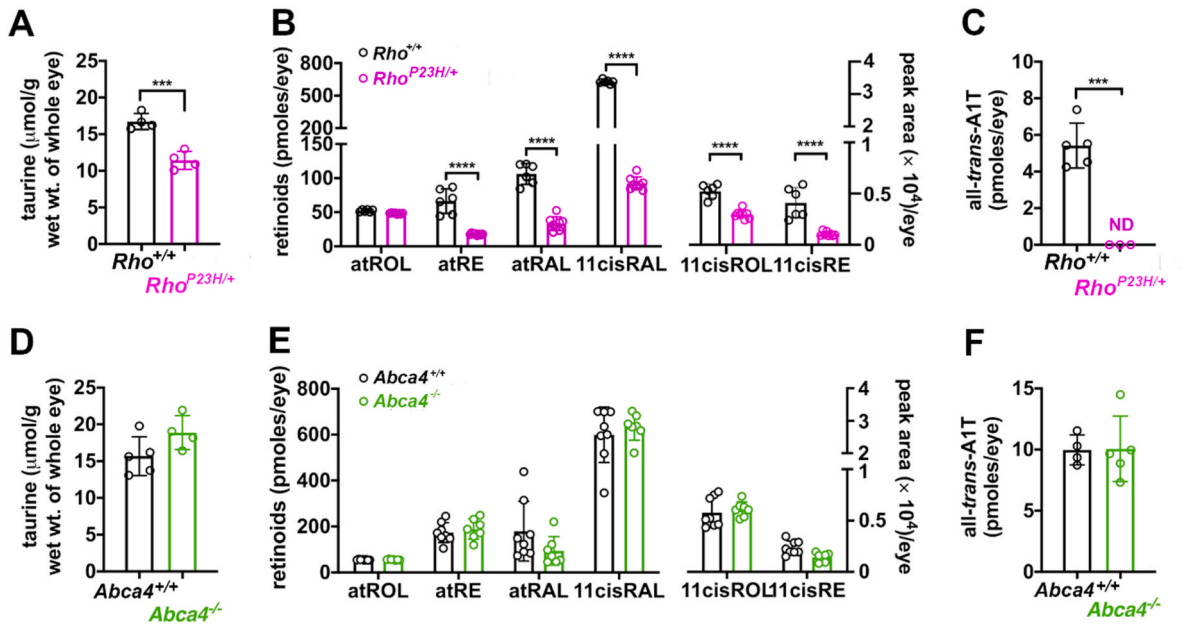

Fig. 5. Quantitation of taurine, retinoids and all-*trans*-A1T in albino $Rho^{P23H/+}$ and albino $Abca4^{-/-}$ mice. Taurine was quantified by HPLC and retinoids and all-*trans*-A1T were quantified by UPLC. Taurine and all-*trans*-A1T were measured in light-adapted mice whereas retinoids were quantified in dark-adapted mice. (A–C) Taurine (A) and retinoids (B) (all-*trans*-retinol, atROL; all-*trans*-retinyl ester, atRE; all-*trans*-retinal, atRAL; 11-*cis*-retinal, 11cisRAL) are depleted together with all-*trans*-A1T (C) in dark-adapted mice heterozygous for the P23H rhodopsin mutation ($Rho^{P23H/+}$ mice) relative to albino $Rho^{+/+}$ mice. Mice were 1 month (retinoid measurement) and 2 months (taurine, A1T) of age. (D–F) Measurement of taurine (D), retinoids (E) and all-*trans*-A1T (F) in albino $Abca4^{-/-}$ mice and $Abca4^{+/+}$ (BALB/cByJ). Age (1–4 months) and gender-matched. Extraction method 1 (B) and method 2 (E). Each plotted value is based on one eye (A, B, D, E) and four eyes (C, F); $n = 4$–8, mean ± SD. P values were determined by two-way ANOVA and Sidak's multiple comparison test (B, E); unpaired t-test (A, C, D, F). ***$P < 0.001$.

samples (4 eyes per sample) of albino $Rho^{P23H/+}$ mice at age 8 weeks, whereas all-*trans*-A1T was detected in age-matched WT mice ($P < 0.001$, unpaired two-tailed t-test) (Fig. 5C).

3.7. Taurine, retinoid and A1T in $Abca4^{-/-}$ mice

The $Abca4^{-/-}$ mouse is a model of recessive Stargardt disease, a disorder of central retina that begins with deterioration of RPE and photoreceptor cells [53]. Impaired ABCA4 activity is associated with less efficient reduction of retinaldehyde to retinol [54] and in albino $Abca4^{-/-}$ mice photoreceptor cell degeneration is readily detectable at age 9 months [40]. Thus we also compared A1T levels in untreated albino $Abca4^{-/-}$ versus WT mice (age 3 months). No differences were observed when eyes were studied at age 3 months (Fig. 5F), an age at which photoreceptor cell loss has not yet occurred. Likewise, levels of retinoids (atROL, atRE, atRAL, 11cisRAL) and taurine were not different in albino $Abca4^{-/-}$ versus WT mice (Fig. 5D and E).

3.8. Decreased cellular taurine by β-alanine-treatment results in reduced bisretinoid levels in agouti and albino $Abca4^{-/-}$ mice

Depletion of ocular taurine by β-alanine resulted in reduced bisretinoid levels in albino and agouti $Abca4^{-/-}$ mice (Fig. 6A–G). In albino $Abca4^{-/-}$ mice housed in cyclic light and treated with β-alanine from 1 to 3 months of age, levels of A2E, A2-GPE, A2-DHP-PE, and atRALdi measured in whole eyes were reduced by 39.3%, 37.6%, 48.1% and 77.8% ($P < 0.01$, 2-way ANOVA and Sidak's multiple comparison test), respectively relative to untreated albino $Abca4^{-/-}$ mice (Fig. 6B). The sum of all bisretinoids in the β-alanine-treated mice was 44.4% lower ($P < 0.01$, unpaired two-tailed t-test) (Fig. 6C). Treatment of agouti $Abca4^{-/-}$ mice with oral GES or β-alanine for 2 months before euthanasia at 3 months also reduced total bisretinoids by 55.2% ($P < 0.05$, unpaired two-tailed t-test) (Figs. 6E) and 32.7%, respectively (not statistically significant, $P > 0.05$) (Fig. 6G). The individual bisretinoid A2E (A2E and isomers) was significantly reduced by 60.7% ($P < 0.05$, 2-way ANOVA and Sidak's multiple comparison test) (Fig. 6D) after GES treatment and was reduced by 44.6% after β-alanine treatment ($P < 0.05$, 2-way ANOVA and Sidak's multiple comparison test) (Fig. 6F).

3.9. Taurine supplementation in mice does not alter bisretinoid levels

To test for an effect of taurine supplementation, we measured bisretinoids in 3 month-old albino $Abca4^{-/-}$ mice after taurine was delivered in drinking water for 2 months. No statistically significant change was observed between control and supplemented mice (Fig. 7A).

Since bisretinoids are the source of fundus autofluorescence (fundus AF) elicited with 488 nm excitation, we also measured bisretinoids in albino $Abca4^{-/-}$ mice by non-invasive quantitative fundus autofluorescence (qAF) [55]. Oral delivery of taurine from age 1–3 months had no effect on qAF levels (Fig. 7B).

3.10. Outer nuclear layer thickness in taurine-deficient mice

Photoreceptor cell viability in albino $Abca4^{-/-}$ mice was assessed by measuring ONL thickness (Fig. 8). Importantly, the thinning of ONL that is indicative of reduced photoreceptor cell viability in albino $Abca4^{-/-}$ mice was more pronounced in mice treated with β-alanine from age 6–8 months mice (Fig. 8A). The ONL area, determined using the sum of ONL thicknesses in superior and inferior retina (2 mm), was decreased by 13% ($P < 0.0001$, unpaired two-tailed t-test) in mice receiving oral β-alanine compared with the control mice (Fig. 8C).

3.11. Quantitation of methylglyoxal-adduct proteins by ELISA

Photooxidation-induced and iron-mediated degradation of bisretinoid leads to the production of carbonyl-carrying molecular fragments such as methylglyoxal (MG) [21,56]. We have previously detected an adduct of MG with arginine residues (MG-derived hydroimidazolone; MG-H1), in posterior eyecups of agouti $Rdh8^{-/-}/Abca4^{-/-}$ mice by indirect competitive ELISA [22] and corroborated this finding by

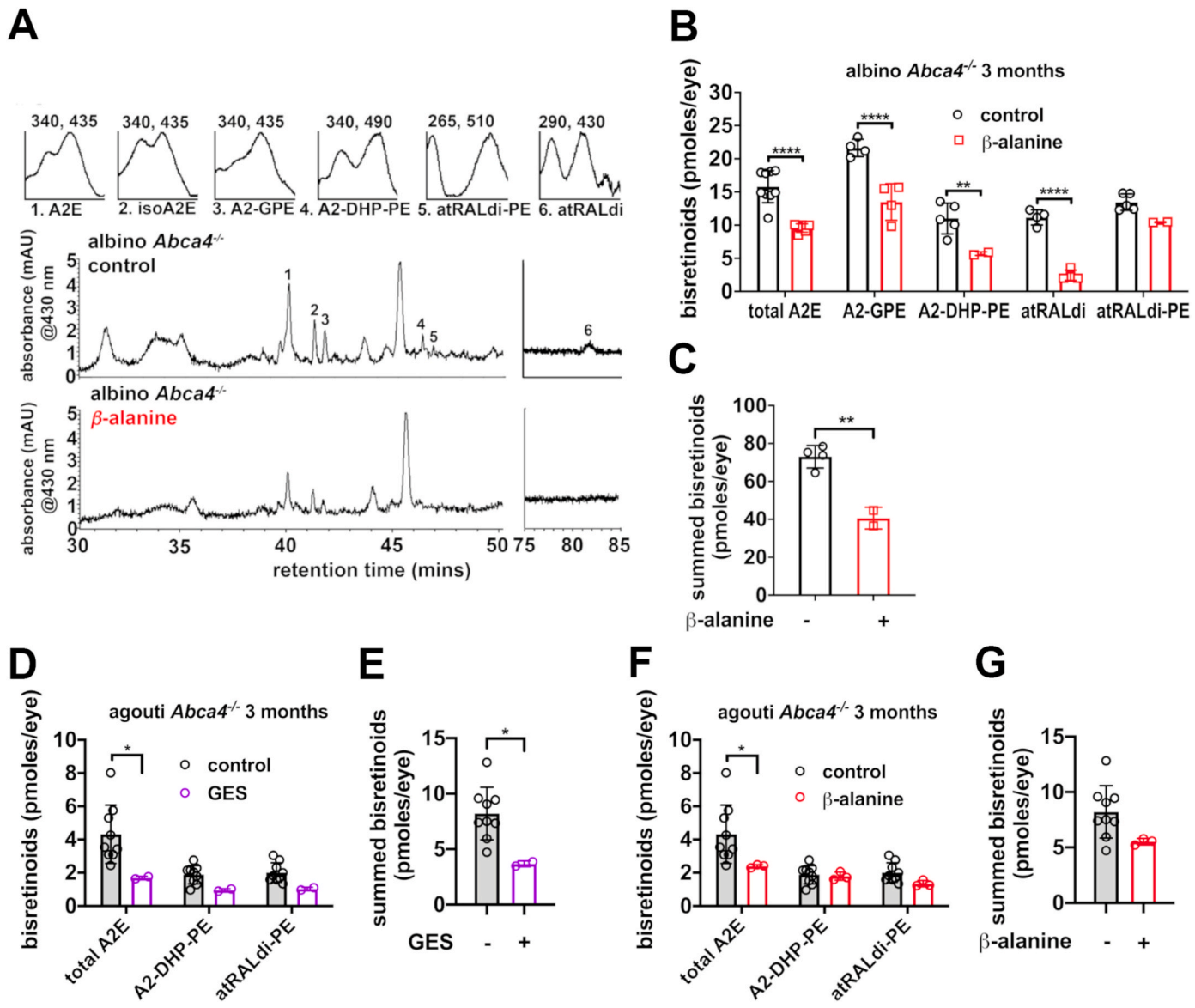

Fig. 6. Reduction of taurine levels by β-alanine and guanidinoethyl sulfonate (GES) is associated with reduced bisretinoid levels in cyclic light-reared *Abca4*$^{-/-}$ mice. Mice were albino and agouti as indicated. (A) Chromatographic detection of bisretinoids. (B–G) HPLC quantitation (B–G): total A2E, A2-DHP-PE, atRAL di-PE; UPLC quantitation (B, C): A2-GPE, atRALdi. Treatment with β-alanine (2% in drinking water) and GES (1% in drinking water) for 2 months (age 1–3 months) as indicated. Extraction method B: A-C; extraction method A: D-G. Individual values are based on 1–2 eyes/sample and 2–8 eyes/samples (D, F). Mean ± SD. *P* values were determined by two-way ANOVA and Sidak's multiple comparison test (B, D, F) and *t*-test (C, E, G); $^{*}P < 0.05$, $^{**}P < 0.01$; $^{****}P < 0.0001$.

UPLC-MS detection of 2,4-dinitrophenylhydrazones (DNPH-derivatized) photocleavage products of the bisretinoid A2E [22]. Here too, we measured the production of MG-derived hydroimidazolone (MG-H1), an adduct of MG with arginine residues in isolated neural retina and RPE by indirect competitive ELISA. In albino *Abca4*$^{-/-}$ mice treated with β-alanine for 2 months MG-H1 was significantly increased by 23.2% in isolated neural retina relative to untreated controls ($P < 0.01$, 2-way ANOVA and Sidak's multiple comparison test) (Fig. 9A). As shown in Supplementary Fig. S2, taurine-MG adducts were not detected in a reaction mixture of MG with taurine. This control indicated that the ELISA assay is specific for MG-H1 adducts.

3.12. *In vitro cellular photooxidation assay*

To corroborate our findings in mice we also tested the effects of taurine in a known cell model using cell viability (MTT assay) as the outcome measure. Specifically, β-alanine (1 mM, 4 treatments over 2 weeks) was delivered to A2E-containing ARPE-19 after which the cells were exposed to 430-nm light to induce A2E photodegradation. Cell viability was reduced by 19% ($P < 0.05$, one-way ANOVA and Newman-Keuls multiple comparison test) (Fig. 9B) due to taurine deficiency. In a control experiment, delivery of β-alanine alone to ARPE-19 cells at a concentration of 0.1–1 mM for 2 weeks (4 treatments) did not cause the death of the cells as shown by MTT assay (Fig. 9D).

We surmised that the absence of an effect of taurine supplementation in the mouse was attributable to taurine sufficiency in the mouse retinae. Indeed taurine-treatment (1 mM) was associated with a 24% increase in viability of ARPE-19 cells burdened by A2E accumulation and blue light exposure ($P < 0.001$, 1-way ANOVA and Tukey's multiple comparison test) (Fig. 9C). Moreover, neither the addition of taurine in acellular assays nor depletion of the latter by treating ARPE-19 cells with the taurine transport inhibitors, GES, attenuated photooxidative loss of A2E, indicating that the effects of taurine are not mediated by inhibiting photooxidation (Supplementary Fig. S3). But more likely occur

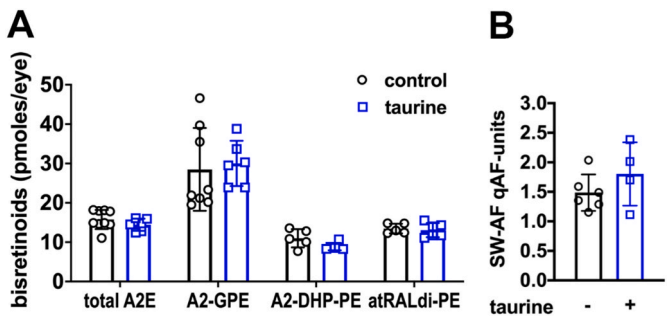

Fig. 7. Supplementation with taurine in drinking water (2.5%) does not afford a difference in bisretinoid levels in albino *Abca4*$^{-/-}$ mice. Mice were treated from age 1–3 months. (A) Quantitation of A2E and isomers; A2-GPE, A2-DHP-PE, and atRALdi-PE. Extraction method B. Individual values are based on 1–2 eyes/sample. Mean ± SD of 5–9 samples. (B) Quantitative fundus autofluororescence units (qAF) (488 nm). Values are mean ± SD of 4–6 mice. *P* values were determined by two-way ANOVA and Sidak's multiple comparison test (A); unpaired *t*-test (B).

downstream at the stage of photodegradation (Supplementary Fig. S4).

4. Discussion

Although A1T was described several years ago, this taurine-retinaldehyde Schiff base has been largely ignored. We approached this study with the knowledge that numerous findings indicate that in retina taurine function primarily relates to photoreceptor cells. In this work we have demonstrated that depletion of taurine by selective inhibition of the taurine transporter TauT leads to reduced taurine in plasma and neural retina, reduced A1T in retina, reduced scotopic and photopic a-wave amplitudes and decreased bisretinoid lipofuscin levels in *Abca4*$^{-/-}$ mice. *β*-alanine-induced taurine deficiency was also associated with elevated MG adducts measured as MG-H1. In albino *Rho*$^{P23H/+}$ knock-in mice we found that at age 1 month 11-*cis*-retinal and all-*trans*-retinal were reduced by 85% and 69%, respectively. The concentration of ocular taurine in albino *Rho*$^{P23H/+}$ mice was decreased by 31.7% when compared to *Rho*$^{+/+}$ control mice and A1T was not detectable. Previous studies in a dog model of RP also showed that taurine was reduced secondary to photoreceptor loss [57]. Likewise, a pronounced decline in taurine accompanies photoreceptor cell degeneration in the postnatal rd1 and C3H mouse [1,58] and photoreceptor cell degeneration induced by N-methyl-N-nitrosourea (MNU) [51]. On the other hand, destruction of inner retina has a minimal effect on the content of taurine in retina [1]. On the basis of our current findings and the previous findings of others, we surmise that with a loss of photoreceptor cells there is little need for taurine and A1T.

We also observed that in untreated *Abca4*$^{-/-}$ mice, there were no differences in ocular taurine, A1T nor retinoid levels as compared to wild-type mice at age 3 months. We note that at the latter age, loss of photoreceptor cells, as reflected in ONL thinning, is not observed [59]. However, ONL thinning that is detectable at 8 months of age in *Abca4*$^{-/-}$ mice was aggravated by *β*-alanine-depletion of taurine. This adverse effect on photoreceptor cells is consistent with previous findings. For instance in cats [60] raised on a diet that depletes taurine in serum and retina [49,50,61] and in mice carrying a homozygous deletion in the SLC6a6Tau gene encoding for TauT, the pronounced depletion of taurine leads to photoreceptor cell degeneration and absent ERG responses after 4 weeks of age [62]. Depletion of taurine (by 1% guanidinoethyl sulfonate) in albino rats is also associated with retinal degeneration that is exacerbated, particularly in central superior retina, by elevated environmental light [63]. In humans, a diet chronically deficient in taurine results in abnormal ERG recordings that can be reversed by intravenous delivery of taurine [64,65].

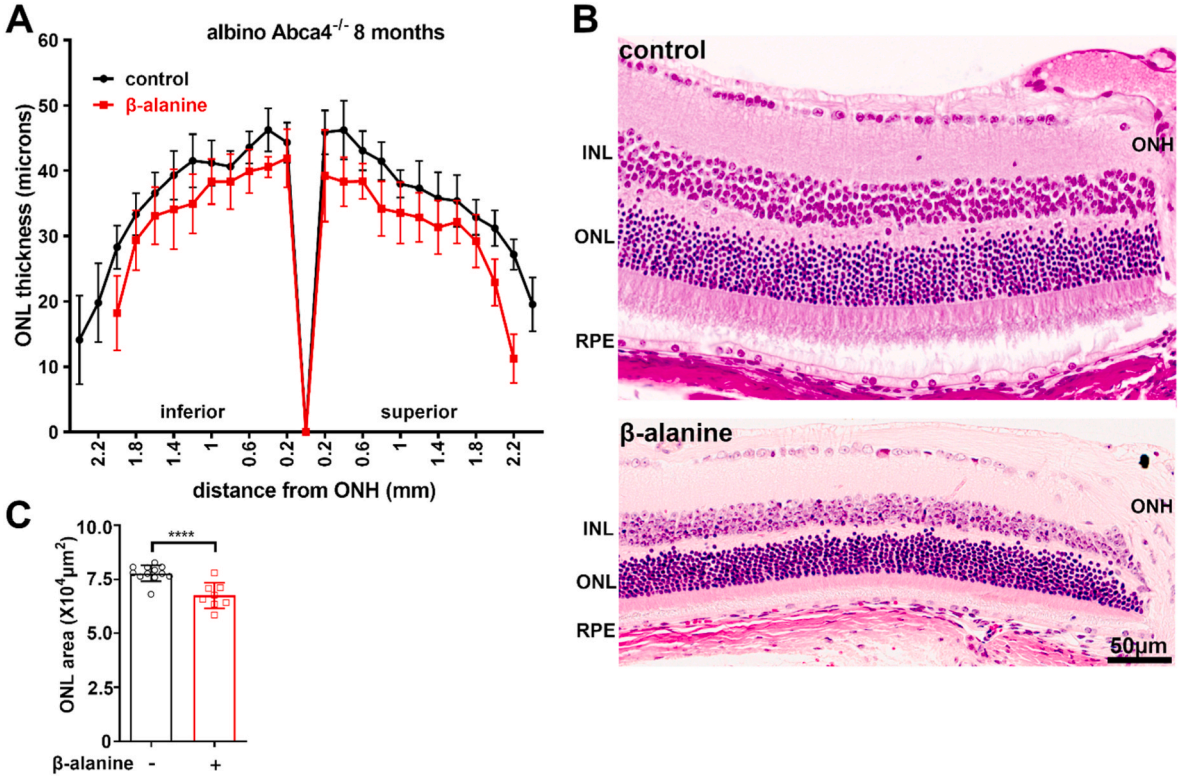

Fig. 8. Photoreceptor cell viability measured as outer nuclear layer (ONL) thickness in albino *Abca4*$^{-/-}$ mice (age 8 months) treated with *β*-alanine for 2 months. Outer nuclear thicknesses (mean ± SD) are plotted as distance from the optic nerve head (ONH) (A). Representative light micrographs of inferior quadrant acquired from albino *Abca4*$^{-/-}$ mice (age 8 months) treated with *β*-alanine for 2 months (*β*-alanine) and untreated *Abca4*$^{-/-}$ mice (control) (B). ONL area was calculated from thicknesses measured at 0.2 mm intervals 1.6 mm superior and inferior to the ONH (C). $n = 6$–11 eyes. Unpaired *t*-test. ****$P < 0.0001$.

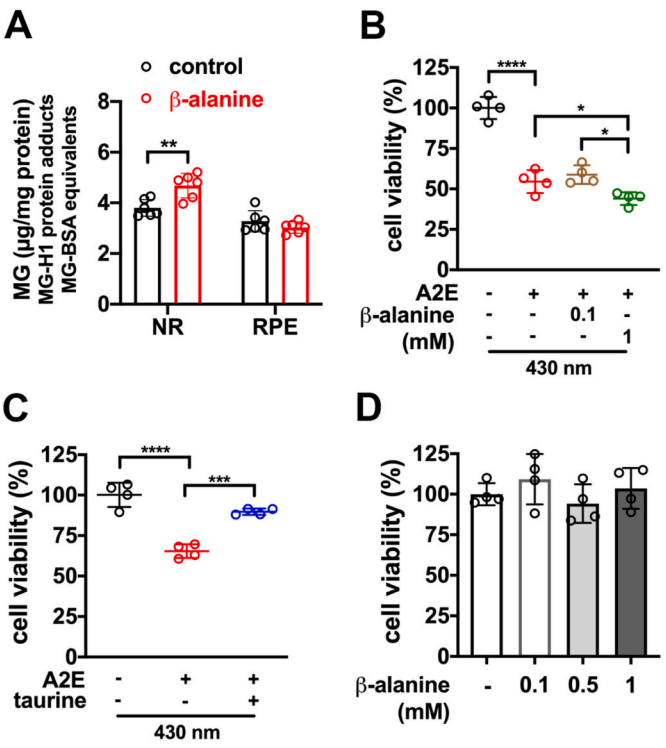

Fig. 9. Quantitation of MG-derived hydroimidazolone (MG-H1) adducts (A) and effect of taurine and *β*-alanine on cell viability in an *in vitro* assay (B–D). (A) MG-BSA equivalents of MG-H1 adducts were measured by competitive indirect ELISA in isolated neural retina and RPE in albino *Abca4*$^{-/-}$ mice treated with *β*-alanine (2% in drinking water) for 2 months (4–6 months) versus untreated control. Mean ± SD of 3 samples assayed in duplicate; 8 eyes/sample. *P* values were determined by two-way ANOVA and Sidak's multiple comparison test; $^{**}P < 0.01$. ARPE-19 cells that had accumulated A2E and had been treated with *β*-alanine (0.1 and 1 mM) (B) and taurine (1 mM) (C) were exposed to 430 nm light for 30 min. (D) Delivery of *β*-alanine (0.1–1 mM) alone to ARPE-19 cells did not cause death of the cells. Viability was determined by MTT. Mean ± SD, $n = 4$. *P* values determined by 1-way ANOVA and either Newman-Keuls or Tukey's multiple comparison test. $^{*}P < 0.05$, $^{****}P < 0.0001$.

Bisretinoid formation in photoreceptor cells occurs due to unchecked retinaldehyde reactivity. Since taurine sequesters retinaldehyde via formation of the Schiff base A1T [16,17] one might have expected to find that taurine supplementation with the associated formation of A1T would reduce unwanted bisretinoid formation. Instead, we found that taurine-treatment had no effect on bisretinoid formation in young *Abca4*$^{-/-}$ mice. Conversely, depletion of ocular taurine by *β*-alanine resulted in reduced bisretinoid levels in albino and agouti *Abca4*$^{-/-}$ mice without a corresponding change in 11-*cis*-retinaldehyde levels. This differs from some other conditions under which reduced bisretinoid formation has been shown to follow from a reduction in serum retinol and 11-*cis*-retinal under light-adapted conditions [66,67]. Moreover, it has previously been discerned that reduced bisretinoid is associated with protection of photoreceptor cell viability [68]. Here however, *β*-alanine-associated reduction in taurine levels also attenuated bisretinoid levels while accentuating ONL thinning in *Abca4*$^{-/-}$ mice. The finding that a decrease in ocular taurine resulted in lower bisretinoid levels may indicate that A1T preserves the aldehyde moiety of 11-*cis*-retinaldehyde. It is the aldehyde group that reacts with PE (2:1 ratio) to form bisretinoid. This scenario may explain the association of diminished A1T with reduced bisretinoid formation.

Oxidative degradation of bisretinoids such as A2E and all-*trans*-retinal dimer, releases a mixture of aldehyde- and dicarbonyl bearing fragments (methylglyoxal, MG; glyoxal, GO) that react with and form adducts (MG-H1; hydroimidazolone) on arginine residues of proteins thereby eliciting molecular damage [69]. Accordingly, we have demonstrated light-mediated carbonyl release from bisretinoid *in vitro* and in the *Abca4*$^{-/-}$ mouse [22,24,70]. We have found that interventions that mitigate the impact of bisretinoid photodegradative products can protect against photoreceptor cell degeneration in mice [21,56]. These conditions were dark-rearing of mice to suppress photodegradative processes [21], treatment with vitamin E that as antioxidant served to reduce photooxidation-associated photodegradation of bisretinoid [21] and iron chelation by deferiprone (DFP) to suppress iron-promoted oxidation based degradation of bisretinoid in mice [56]. In the studies reported here, *β*-alanine-induced taurine deficiency was associated with an increase in MG adducts measured as MG-H1. This finding suggests that taurine scavenges these dicarbonyls (Fig. S5) and that like glutathione [71] taurine may be bifunctional in photoreceptor cells. Scavenging activity such as this is also consistent with high taurine concentrations in retina [2].

As opposed to alleviating the adverse consequences of bisretinoid degradation we found in the current work that *β*-alanine-associated taurine-deficiency resulted in an increase in MG-H1 adducts in neural retina. These findings suggest that normally taurine serves to neutralize MG fragments perhaps by direct quenching reactions between taurine and dicarbonyl species released by bisretinoid photodegradation [72]. Loss of this protection could account for the reduction in photoreceptor cell viability evidenced by ONL thinning.

Aside from retina, taurine is reported to have myriad effects suggesting a more generalized role in metabolic processes. For instance, taurine has been shown to participate in osmoregulation and cell volume regulation [73] and in the formation of bile acids such as tauroursodeoxycholic acid [74]. The latter is reported to increase the survival of photoreceptor cells in rd10 mice [75]. Taurine has been shown to play a role in the stabilization of mitochondrial gene translation. Specifically, taurine is a constituent of post-transcriptionally modified uridine nucleotides (5-taurinomethyluridine moieties) in select human mitochondrial transfer RNAs (mt-tRNA), the *β*-carbon of L-serine being the source of the methylene group [76]. Inadequate taurine-based modification results in impaired expression of respiratory chain enzymes and thus defective oxidative phosphorylation [77,78]. Also of interest is evidence indicating that taurine modulates the phospholipid content of membrane by reducing the activity of the methyltransferase that converts phosphatidylethanolamine to phosphatidylcholine [42,79].

5. Conclusion

While taurine may be multi-functional, the reversible Schiff base A1T likely has visual cycle retinoids as its focus. A1T is an amphiphilic small molecule carrying a negatively charged sulfonated group together with a hydrophobic retinoid moiety. Because of its hydrophilic moiety, A1T has the potential to reside in a milieu distinct from NRPE. Engagement of A1T in a cone-driven visual cycle could account for the low levels of 11-*cis*-A1T in mouse since in this rodent only 5% of photoreceptor cells are cones. If A1T constitutes a pool of mobilizable 11-*cis*-retinal as we have suggested [17], a *β*-alanine-induced deficit in taurine could reduce the availability of retinaldehyde, an effect that would manifest as diminished ERG responses. Reduced visual chromophores may account for ONL thinning and would attenuate bisretinoid formation in photoreceptor cells. That said, it is also possible that the effect of diminished taurine levels on photoreceptor cell viability involves an additional mechanism. Specifically taurine may serve to scavenge dicarbonyls released by bisretinoid photodegradation.

Data availability

All relevant data are provided in the paper and supporting information file.

Fundings

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Declaration of competing interest

Authors (Hye Jin Kim, Jin Zhao, and Janet R. Sparrow) do not have conflicts of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.redox.2022.102386.

References

[1] H. Ripps, W. Shen, Review: taurine: a "very essential" amino acid, Mol. Vis. 18 (2012) 2673–2686.
[2] N. Froger, et al., Taurine: the comeback of a neutraceutical in the prevention of retinal degenerations, Prog. Retin. Eye Res. 41 (2014) 44–63.
[3] J.R. Chao, et al., Human retinal pigment epithelial cells prefer proline as a nutrient and transport metabolic intermediates to the retinal side, J. Biol. Chem. 292 (2017) 12895–12905.
[4] K.E. Smith, et al., Cloning and expression of a high affinity taurine transporter from rat brain, Mol. Pharmacol. 42 (1992) 563–569.
[5] M.N. Preising, et al., Biallelic mutation of human SLC6A6 encoding the taurine transporter TAUT is linked to early retinal degeneration, Faseb. J. 33 (2019) 11507–11527.
[6] S. Vinnakota, X. Qian, H. Egal, V. Sarthy, H.K. Sarkar, Molecular characterization and in situ localization of a mouse retinal taurine transporter, J. Neurochem. 69 (1997) 2238–2250.
[7] H. Pasantes-Morales, O. Quesada, A. Carabez, R.J. Huxtable, Effects of the taurine transport antagonist, guanidinoethane sulfonate, and beta-alanine on the morphology of rat retina, J. Neurosci. Res. 9 (1983) 135–143.
[8] M.S. Starr, M.J. Voaden, The uptake, metabolism and release of 14C-taurine by rat retina in vitro, Vis. Res. 12 (1972) 1261–1269.
[9] C. Tiruppathi, M. Brandsch, Y. Miyamoto, V. Ganapathy, F.H. Leibach, Constitutive expression of the taurine transporter in a human colon carcinoma cell line, Am. J. Physiol. 263 (1992) G625–G631.
[10] Z. Zhang, et al., Taurine supplementation reduces oxidative stress and protects the liver in an iron-overload murine model, Mol. Med. Rep. 10 (2014) 2255–2262.
[11] R. Dawson Jr., S. Liu, B. Eppler, T. Patterson, Effects of dietary taurine supplementation or deprivation in aged male Fischer 344 rats, Mech. Ageing Dev. 107 (1999) 73–91.
[12] M. Ansar, et al., Taurine treatment of retinal degeneration and cardiomyopathy in a consanguineous family with SLC6A6 taurine transporter deficiency, Hum. Mol. Genet. 29 (2020) 618–623.
[13] O. Quesada, A. Picones, H. Pasantes-Morales, Effect of light deprivation on the ERG responses of taurine-deficient rats, Exp. Eye Res. 46 (1988) 13–20.
[14] R.N. Rasmussen, C. Lagunas, J. Plum, R. Holm, C.U. Nielsen, Interaction of GABA-mimetics with the taurine transporter (TauT, Slc6a6) in hyperosmotic treated Caco-2, LLC-PK1 and rat renal SKPT cells, Eur. J. Pharmaceut. Sci. 82 (2016) 138–146.
[15] F. Jammoul, et al., Taurine deficiency damages photoreceptors and retinal ganglion cells in vigabatrin-treated neonatal rats, Mol. Cell. Neurosci. 43 (2010) 414–421.
[16] A.M. Petrosian, et al., New HPLC evidence on endogenous tauret in retina and pigment epithelium, Adv. Exp. Med. Biol. 483 (2000) 453–460.
[17] H.J. Kim, J. Zhao, J.R. Sparrow, Vitamin A aldehyde-taurine adduct and the visual cycle, Proc. Natl. Acad. Sci. U. S. A. 117 (2020) 24867–24875.
[18] J.C. Saari, D.L. Bredberg, Photochemistry and stereoselectivity of cellular retinaldehyde-binding protein from bovine retina, J. Biol. Chem. 262 (1987) 7618–7622.
[19] R. Parker, J.S. Wang, V.J. Kefalov, R.K. Crouch, Interphotoreceptor retinoid-binding protein as the physiologically relevant carrier of 11-cis-retinol in the cone visual cycle, J. Neurosci. 31 (2011) 4714–4719.
[20] J.C. Saari, J.W. Crabb, Focus on molecules: cellular retinaldehyde-binding protein (CRALBP), Exp. Eye Res. 81 (2005) 245–246.
[21] K. Ueda, J. Zhao, H.J. Kim, J.R. Sparrow, Photodegradation of retinal bisretinoids in mouse models and implications for macular degeneration, Proc. Natl. Acad. Sci. USA 113 (2016) 6904–6909.
[22] J. Zhou, K. Ueda, J. Zhao, J.R. Sparrow, Correlations between photodegradation of bisretinoid constituents of retina and dicarbonyl adduct deposition, J. Biol. Chem. 290 (2015) 27215–27227.
[23] K. Yamamoto, K.D. Yoon, K. Ueda, M. Hashimoto, J.R. Sparrow, A novel bisretinoid of retina is an adduct on glycerophosphoethanolamine, Invest. Ophthalmol. Vis. Sci. 52 (2011) 9084–9090.
[24] Y. Wu, E. Yanase, X. Feng, M.M. Siegel, J.R. Sparrow, Structural characterization of bisretinoid A2E photocleavage products and implications for age-related macular degeneration, Proc. Natl. Acad. Sci. USA 107 (2010) 7275–7280.
[25] S. Sakami, et al., Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations, J. Biol. Chem. 286 (2011) 10551–10567.
[26] M.J. Mattapallil, et al., The Rd8 mutation of the Crb1 gene is present in vendor lines of C57BL/6N mice and embryonic stem cells, and confounds ocular induced mutant phenotypes, Invest. Ophthalmol. Vis. Sci. 53 (2012) 2921–2927.
[27] C.A. Parish, M. Hashimoto, K. Nakanishi, J. Dillon, J.R. Sparrow, Isolation and one-step preparation of A2E and iso-A2E, fluorophores from human retinal pigment epithelium, Proc. Natl. Acad. Sci. U. S. A. 95 (1998) 14609–14613.
[28] J.R. Sparrow, et al., Involvement of oxidative mechanisms in blue-light-induced damage to A2E-laden RPE, Invest. Ophthalmol. Vis. Sci. 43 (2002) 1222–1227.
[29] Y. Wu, N.E. Fishkin, A. Pande, J. Pande, J.R. Sparrow, Novel lipofuscin bisretinoids prominent in human retina and in a model of recessive Stargardt disease, J. Biol. Chem. 284 (2009) 20155–20166.
[30] N. Fishkin, J.R. Sparrow, R. Allikmets, K. Nakanishi, Isolation and characterization of a retinal pigment epithelial cell fluorophore: an all-trans-retinal dimer conjugate, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 7091–7096.
[31] A.M. Petrosian, J.E. Haroutounian, L.V. Zueva, Tauret. A taurine-related endogenous substance in the retina and its role in vision, Adv. Exp. Med. Biol. 403 (1996) 333–342.
[32] M.A. Kane, A.E. Folias, J.L. Napoli, HPLC/UV quantitation of retinal, retinol, and retinyl esters in serum and tissues, Anal. Biochem. 378 (2008) 71–79.
[33] V.C. Greenstein, et al., Quantitative fundus autofluorescence in HCQ retinopathy, Invest. Ophthalmol. Vis. Sci. 61 (2020) 41.
[34] J.R. Sparrow, et al., Fundus autofluorescence and the bisretinoids of retina, Photochem. Photobiol. Sci. 9 (2010) 1480–1489.
[35] S.R. Kim, et al., The Rpe65 Leu450Met variant is associated with reduced levels of the RPE lipofuscin fluorophores A2E and iso-A2E, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 11668–11672.
[36] X. Wang, D. Chi, G. Su, L. Li, L. Shao, Determination of taurine in biological samples by high-performance liquid chromatography using 4-fluoro-7-nitrobenzofurazan as a derivatizing agent, Biomed. Environ. Sci. 24 (2011) 537–542.
[37] M. Ogasawara, T. Nakamura, I. Koyama, M. Nemoto, T. Yoshida, Reactivity of taurine with aldehydes and its physiological role, Adv. Exp. Med. Biol. 359 (1994) 71–78.
[38] J. Zhao, K. Ueda, M. Riera, H.J. Kim, J.R. Sparrow, Bisretinoids mediate light sensitivity resulting in photoreceptor cell degeneration in mice lacking the receptor tyrosine kinase Mer, J. Biol. Chem. 293 (2018) 19400–19410.
[39] L. Wu, T. Nagasaki, J.R. Sparrow, Photoreceptor cell degeneration in *Abcr*$^{-/-}$ mice, Adv. Exp. Med. Biol. 664 (2010) 533–539.
[40] J.R. Sparrow, et al., Quantitative fundus autofluorescence in mice: correlation with HPLC quantitation of RPE lipofuscin and measurement of retina outer nuclear layer thickness, Invest. Ophthalmol. Vis. Sci. 54 (2013) 2812–2820.
[41] P.F. Canellas, A.E. Karu, Statistical package for analysis of competition ELISA results, J. Immunol. Methods 47 (1981) 375–385.
[42] R.J. Huxtable, S. Crosswell, D. Parker, Phospholipid composition and taurine content of synaptosomes in developing rat brain, Neurochem. Int. 15 (1989) 233–238.
[43] C.C. Bridges, et al., Regulation of taurine transporter expression by NO in cultured human retinal pigment epithelial cells, Am. J. Physiol. Cell Physiol. 281 (2001) C1825–C1836.
[44] D.C. Hood, D.G. Birch, Assessing abnormal rod photoreceptor activity with the a-wave of the electroretinogram: applications and methods, Doc. Ophthalmol. 92 (1996) 253–267.
[45] W. Hadj-Said, et al., Quantitative and topographical analysis of the losses of cone photoreceptors and retinal ganglion cells under taurine depletion, Invest. Ophthalmol. Vis. Sci. 57 (2016) 4692–4703.
[46] A. Adekeye, M. Haeri, E. Solessio, B.E. Knox, Ablation of the proapoptotic genes CHOP or Ask 1 does not prevent or delay loss of visual function in a P23H transgenic mouse model of retinitis pigmentosa, PLoS One 9 (2014), e83871.
[47] T.P. Dryja, et al., A point mutation of the rhodopsin gene in one form of retinitis pigmentosa, Nature 343 (1990) 364–366.
[48] X. Liu, P. Garriga, H.G. Khorana, Structure and function in rhodopsin: correct folding and misfolding in two point mutants in the intradiscal domain of rhodopsin identified in retinitis pigmentosa, Proc. Natl. Acad. Sci. U. S. A. 93 (1996) 4554–4559.
[49] E.L. Berson, K.C. Hayes, A.R. Rabin, S.Y. Schmidt, G. Watson, Retinal degeneration in cats fed casein. II. Supplementation with methionine, cysteine, or taurine, Invest. Ophthalmol. 15 (1976) 52–58.
[50] S.Y. Schmidt, E.L. Berson, K.C. Hayes, Retinal degeneration in the taurine-deficient cat, Trans. Sect. Ophthalmol. Am. Acad. Ophtalmol. Otolaryngol. 81 (1976) OP687–693.
[51] Y. Tao, et al., Systemic taurine treatment provides neuroprotection against retinal photoreceptor degeneration and visual function impairments, Drug Des. Dev. Ther. 13 (2019) 2689–2702.
[52] D. Gaucher, et al., Taurine deficiency damages retinal neurones: cone photoreceptors and retinal ganglion cells, Amino Acids 43 (2012) 1979–1993.
[53] G.A. Fishman, et al., Variation of clinical expression in patients with Stargardt dystrophy and sequence variations in the ABCR gene, Arch. Ophthalmol. 117 (1999) 504–510.
[54] Y. Tsybovsky, R.S. Molday, K. Palczewski, The ATP-binding cassette transporter ABCA4: structural and functional properties and role in retinal disease, Adv. Exp. Med. Biol. 703 (2010) 105–125.

[55] J.R. Sparrow, J.E. Dowling, D. Bok, Understanding RPE lipofuscin, Invest. Ophthalmol. Vis. Sci. 54 (2013) 8325–8326.
[56] K. Ueda, et al., Iron promotes oxidative cell death caused by bisretinoids of retina, Proc. Natl. Acad. Sci. U. S. A. 115 (2018) 4963–4968.
[57] S.Y. Schmidt, G.D. Aguirre, Reductions in taurine secondary to photoreceptor loss in Irish setters with rod-cone dysplasia, Invest. Ophthalmol. Vis. Sci. 26 (1985) 679–683.
[58] H.T. Orr, A.I. Cohen, J.A. Carter, The levels of free taurine, glutamate, glycine and gamma-amino butyric acid during the postnatal development of the normal and dystrophic retina of the mouse, Exp. Eye Res. 23 (1976) 377–384.
[59] L. Wu, K. Ueda, T. Nagasaki, J.R. Sparrow, Light damage in Abca4 and Rpe65rd12 mice, Investig. Ophthalmol. Vis. Sci. 55 (2014) 1910–1918.
[60] S.G. Jacobson, C.M. Kemp, F.X. Borruat, M.H. Chaitin, D.J. Faulkner, Rhodopsin topography and rod-mediated function in cats with the retinal degeneration of taurine deficiency, Exp. Eye Res. 45 (1987) 481–490.
[61] G.D. Aguirre, Retinal degeneration associated with the feeding of dog foods to cats, J. Am. Vet. Med. Assoc. 172 (1978) 791–796.
[62] B. Heller-Stilb, et al., Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice, Faseb. J. 16 (2002) 231–233.
[63] L.M. Rapp, L.A. Thum, R.E. Anderson, Synergism between environmental lighting and taurine depletion in causing photoreceptor cell degeneration, Exp. Eye Res. 46 (1988) 229–238.
[64] M.E. Ament, H.S. Geggel, J.R. Heckenlively, D.A. Martin, J. Kopple, Taurine supplementation in infants receiving long-term total parenteral nutrition, J. Am. Coll. Nutr. 5 (1986) 127–135.
[65] H.S. Geggel, M.E. Ament, J.R. Heckenlively, D.A. Martin, J.D. Kopple, Nutritional requirement for taurine in patients receiving long-term parenteral nutrition, N. Engl. J. Med. 312 (1985) 142–146.
[66] N. Dobri, et al., A1120, a nonretinoid RBP4 antagonist, inhibits formation of cytotoxic bisretinoids in the animal model of enhanced retinal lipofuscinogenesis, Invest. Ophthalmol. Vis. Sci. 54 (2013) 85–95.
[67] R.A. Radu, et al., Treatment with isotretinoin inhibits lipofuscin and A2E accumulation in a mouse model of recessive Stargardt's macular degeneration, Proc. Natl. Acad. Sci. U. S. A. 100 (2003) 4742–4747.
[68] C. Bavik, et al., Visual cycle modulation as an approach toward preservation of retinal integrity, PLoS One 10 (2015), e0124940.
[69] N. Rabbani, P.J. Thornalley, Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease, Biochem. Biophys. Res. Commun. 458 (2015) 221–226.
[70] K.D. Yoon, K. Yamamoto, K. Ueda, J. Zhou, J.R. Sparrow, A novel source of methylglyoxal and glyoxal in retina: implications for age-related macular degeneration, PLoS One 7 (2012), e41309.
[71] P.G. Sreekumar, D.A. Ferrington, R. Kannan, Glutathione metabolism and the novel role of mitochondrial GSH in retinal degeneration, Antioxidants 10 (2021).
[72] G. Li, T. Tang, M. Peng, H. He, D. Yin, Direct reaction of taurine with malondialdehyde: evidence for taurine as a scavenger of reactive carbonyl species, Redox Rep. 15 (2010) 268–274.
[73] A. El-Sherbeny, et al., Osmoregulation of taurine transporter function and expression in retinal pigment epithelial, ganglion, and muller cells, Invest. Ophthalmol. Vis. Sci. 45 (2004) 694–701.
[74] C.N. Falany, M.R. Johnson, S. Barnes, R.B. Diasio, Glycine and taurine conjugation of bile acids by a single enzyme. Molecular cloning and expression of human liver bile acid CoA:amino acid N-acyltransferase, J. Biol. Chem. 269 (1994) 19375–19379.
[75] M.J. Phillips, et al., Tauroursodeoxycholic acid preservation of photoreceptor structure and function in the rd10 mouse through postnatal day 30, Invest. Ophthalmol. Vis. Sci. 49 (2008) 2148–2155.
[76] K. Asano, et al., Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease, Nucleic Acids Res. 46 (2018) 1565–1583.
[77] R.J. Morscher, et al., Mitochondrial translation requires folate-dependent tRNA methylation, Nature 554 (2018) 128–132.
[78] S. Schaffer, H.W. Kim, Effects and mechanisms of taurine as a therapeutic agent, Biomol Ther (Seoul) 26 (2018) 225–241.
[79] T. Hamaguchi, J. Azuma, S. Schaffer, Interaction of taurine with methionine: inhibition of myocardial phospholipid methyltransferase, J. Cardiovasc. Pharmacol. 18 (1991) 224–230.