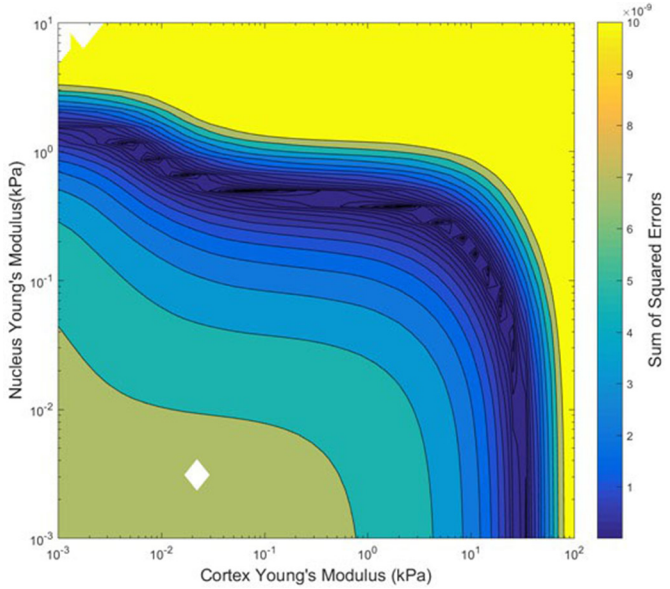
381 Lens Physiology and Biomechanics II
Tuesday, May 09, 2017 3:45 PM–5:30 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 3631–3645/B0190–B0204
Organizing Section: Lens

Program Number: 3631 **Poster Board Number:** B0190
Presentation Time: 3:45 PM–5:30 PM
Cortical cataracts: The case for mechanical stress
Ralph Michael[1], Justin Christopher D'Antin[1], Laura Pinilla Cortés[1], Luis Pareja Aricó[1], Rafael I. Barraquer[1, 2]. [1]Institut Universitari Barraquer, Universitat Autonoma de Barcelona, Barcelona, Spain; [2]Centro de Oftalmología Barraquer, Universitat Internacional de Catalunya, Barcelona, Spain.
Purpose: It is known, that human cortical or cuneiform opacities are accompanied by changes in lens fiber structure and architecture mainly in the equatorial border zone between the lens nucleus and cortex. Because the lens cortex and nucleus have different viscoelastic properties in young and old lenses, we hypothesized that external forces during accommodation cause shear stress predominantly at this nucleus-cortex interface and induce cortical cataracts.
Methods: Experimentally, we studied 5 human donor lenses in a stretching device for anterior eye segments, measuring the forces and obtaining frontal images during stretching. In a literature study, we searched for a correlation between the degree of accommodation, values of refraction and the incidence of different cataract types.
Results: Lenses with cortical cataracts showed ruptures at the nucleus-cortex interface adjacent to the cortical cataracts. Lens thickness did not changed during stretching. Lenses from pre-presbyopic donors showed no ruptures, but changed in thickness. Forces applied were of similar magnitude for all lenses tested (50 to 70 mN). Population-based studies showed that myopes (who accommodate less) have a lower risk of developing cortical than nuclear cataract. Hyperopes (who accommodate more) showed a higher risk of developing cortical than nuclear cataract. A comparative study of refraction before cataract surgery revealed that eyes with cortical cataract had a mean refraction of +2.75 and with nuclear cataract -3.75 diopters.
Conclusions: The ex vivo experiments indicate that the nucleus-cortex interface is vulnerable and can be separated when external forces are applied. According to literature studies, subjects more prone to accommodate developed more cortical cataract. Mechanical stress in the lens induced by accommodation may contribute to the formation of cortical cataract.
Commercial Relationships: Ralph Michael, None; **Justin Christopher D'Antin**, None; **Laura Pinilla Cortés**, None; **Luis Pareja Aricó**, None; **Rafael I. Barraquer**, None

Program Number: 3632 **Poster Board Number:** B0191
Presentation Time: 3:45 PM–5:30 PM
Best Practices for Estimating Lens Mechanical Properties Using a Compression Test
Matthew A. Reilly[1, 2], Andre Cleaver[3], Archit Rede[1], Luis Rodriguez[4], Gabriela Rice[5]. [1]Biomedical Engineering, The Ohio State University, Columbus, OH; [2]Ophthalmology and Visual Science, The Ohio State University, Columbus, OH; [3]Mechanical Engineering, Tufts University, Medford, MA; [4]Biomedical Engineering, University of Texas Health Science Center at San Antonio, San Antonio, TX; [5]Biology, University of Texas at San Antonio, San Antonio, TX.
Purpose: The lens compression test is rapidly gaining in popularity for determining the relative stiffness of mouse lenses. The objective of this study was to determine whether the lens compression test could be used to estimate the intrinsic mechanical properties of the lens.
Methods: A custom lens compression apparatus was developed to allow simultaneous measurements of compressive displacement, force, and lens shape. A mechanical model of lens compression was developed and solved using an inverse finite element method. This model simulated contact between the compression plates and the lens, as well as adhesive and friction effects. This allowed the estimation of the Young's modulus distribution within the lens. Mechanically homogeneous hydrogels were evaluated to validate the experimental and theoretical approaches. Encapsulated and decapsulated porcine lenses were also evaluated for comparison against published modulus values. Stiffness and resilience metrics were also computed according to published methods to evaluate their correlation with intrinsic mechanical properties.
Results: The inverse finite element method was successfully used to extract the modulus of homogeneous hydrogels from a compression test within 5% of the value obtained from a spinning test. Results for the porcine lenses were mixed: it was discovered that matching the force-displacement curve with the model yielded non-unique solutions (Fig. 1). Matching the experimentally-determined geometry overcame this difficulty.
Conclusions: The compression test coupled with the inverse model is useful for estimating the intrinsic mechanical properties of lenses. Stiffness and resilience as calculated in previous studies depend on lens size, shape, and adhesivity and may therefore not strictly correlate with the Young's modulus.



Contour map showing the sum of squared errors between the experimental and model-predicted force-displacement curves at various combinations of Young's modulus for the cortex and nucleus of a 17-week-old mouse lens. Note that a minimum error is not achieved at any one unique pairing of modulus values; rather, a curve corresponding to infinitely many combinations of modulus values yielding minimized errors. White spaces in the figure correspond to modulus pairs for which the model was unable to achieve a solution.
Commercial Relationships: Matthew A. Reilly, None; **Andre Cleaver**, None; **Archit Rede**, None; **Luis Rodriguez**, None; **Gabriela Rice**, None

Program Number: 3633 **Poster Board Number:** B0192
Presentation Time: 3:45 PM–5:30 PM
Patterns of accommodation in natural anisometropia
Apoorva Karsolia, Lawrence R. Stark. Marshall B Ketchum University, Fullerton, CA.
Purpose: Accommodation in anisometropia may respond consensually and produce a yoked accommodative response, or respond independently leading to asymmetrical accommodation responses (anisoaccommodation). A prospective, randomized study was conducted to examine the effect of conflicting accommodative stimuli in *anisometropic individuals* (inter-ocular difference ≥ 1.5 D) and to assess the effects of viewing (binocular or monocular), anisometropia level, test distance and time on the patterns of the accommodative response.
Methods: Dynamic accommodation responses were measured in 11 young, anisometropic subjects (including 3 anisometropic amblyopes) with the Grand Seiko WAM 500 Autorefractor. In the 2 sessions, the viewing condition (monocular or binocular; direct or consensual), the testing distance (400 cm, 38.6 cm and 20.5 cm) and anisometropia level (corrected or uncorrected) were randomized for each subject. In each dynamic trial, accommodation at the 5^{th} ±0.5 second and 20^{th} ±0.5 second was analysed to understand the patterns of the accommodative response. A within-subjects analysis of variance (ANOVA) assessed the factors in the model.
Results: A significant effect of target distance, level of anisometropia and response pattern was found in the group ($p < 0.0001$) for both right and left eyes. Time as a factor was not statistically significant. Post hoc Dunnett analysis showed that the patterns (or strategies) of greatest accommodation effort, average accommodative effort, ocular dominance driven, and complete anisoaccommodation were not significantly different from the actual binocular responses. The mean anisoaccommodative gain after regression analysis was –0.027 ($p = 0.07$), which was not statistically significant.
Conclusions: Natural anisometropes did not demonstrate an ability to anisoaccommodate to anisoaccommodative stimuli. Time as a factor was not statistically significant. The greatest accommodative effort, average accommodation, ocular dominance driven response, and complete anisoaccommodation models were not significantly different from actual binocular responses. Some hypothesized patterns may have produced a mixture of good and poor predictions, thus raising variance and lowering statistical power. Alternatively, it may be that four subgroups of individuals follow each pattern, or that individuals switch between the patterns over time.
Commercial Relationships: Apoorva Karsolia, None; **Lawrence R. Stark**, None

Program Number: 3634 **Poster Board Number:** B0193
Presentation Time: 3:45 PM–5:30 PM
Simulating crystalline lens accommodation ex vivo without scleral support
Caroline Dong[1], Andres Bernal[2], Giuliano Scarcelli[1]. [1]University of Maryland, College Park, Columbia, MD; [2]BIONIKO, Miami, FL.
Purpose: To analyze the accommodation mechanism of the crystalline lens *ex vivo* using a manual lens stretcher that does not require scleral support.
Methods: Crystalline lenses were isolated from 10 fresh porcine eyeballs and from 2 posterior poles of cadaver human eyes (Age 21 and 60). Lenses were mounted onto a manual lens stretcher (Bioniko, Miami Fl) directly at the zonules. Pictures were taken of the stretched and unstretched conditions and uploaded into ImageJ. The diameter of the lens was measured and analyzed in MATLAB in both conditions.
Results: The analysis of porcine lenses revealed the instrument and protocol is robust and reliable. The variability of the measurements between lenses is likely due to inconsistencies with mounting the lens on the device as well as the condition of the lens. Analysis of the young human lens showed an increase in diameter of 0.45 ± 0.27 mm in the stretched condition compared to the unstretched condition, while the old lens showed no change (0.01 ± 0.2 mm).
Conclusions: The lens stretcher can be used to measure the accommodative power of lenses without need of scleral support as in previous lens stretchers. This provides an efficient solution to study accommodation because it mimics more closely the physiological ability of the lens to accommodate, and because, by eliminating the need of the whole eyeball, it reduces the cost of donor tissue and increases its availability.
Commercial Relationships: Caroline Dong, None; **Andres Bernal**, BIONIKO (I); **Giuliano Scarcelli**, University of Maryland, College Park (P), MGH (P)
Support: National Science FoundationCMMI-1537027

Program Number: 3635 **Poster Board Number:** B0194
Presentation Time: 3:45 PM–5:30 PM
Myo/Nog Cells are Present on the Zonules Between the Ciliary Body and Lens
Jacquelyn V. Gerhart[1], Paul G. FitzGerald[2], Robert Getts[3], Liliana Werner[4], Nick Mamalis[4], Mindy George-Weinstein[1].
[1]Research, Phila. College of Osteopathic Medicine, Wynnewood, PA; [2]UC DAvis, Davis, CA; [3]Genisphere, LLC, Hatfield, PA; [4]John Moran Center, University of Utah, Salt Lake City, UT.
Purpose: Myo/Nog cells, identified by their expression of the skeletal muscle specific transcription factor MyoD, the bone morphogenetic inhibitor Noggin and cell surface protein recognized by the G8 monoclonal antibody (mAb), are present throughout the eye. These cells are critical for normal eye development and quickly respond to wounding in the adult lens and retina. In the lens, Myo/Nog cells are a source of contractile myofibroblasts that contribute to the fibrotic form of posterior capsule opacification (PCO), a vision impairing condition that may occur after cataract surgery. In this study, we examined whether Myo/Nog cells may migrate between the ciliary body and lens on the zonules which hold the lens in place and transmit the force that alters the shape of the lens during accommodation.
Methods: Whole eyes or the anterior segments of the eyes from mice, rabbits and humans were fixed, embedded in paraffin and sectioned. Some sections were from rabbit eyes that had undergone cataract surgery. Tissue sections were immunofluorescently labeled with antibodies to G8, Noggin and alpha smooth muscle actin (α-SMA).
Results: Myo/Nog cells were present in the ciliary body and anterior, equatorial and bow regions of the lens in mice and humans. A few Myo/Nog cells were found on the zonules at various distances from the ciliary body. One month following cataract surgery in the rabbit, Myo/Nog cells overlaid wrinkles on the capsule. Myo/Nog cells also were observed on the zonules and within the ciliary body. Some Myo/Nog cells on the zonules expressed the myofibrobast marker α-SMA.
Conclusions: Myo/Nog cells appear to migrate on the zonules. It remains to be determined whether Myo/Nog cells originating in the ciliary body actually penetrate the lens capsule and if their migration is bidirectional. The possibility that the ciliary body is a source of Myo/Nog cells in the lens should be considered when designing therapeutic approaches that target these cells to prevent PCO.
Commercial Relationships: Jacquelyn V. Gerhart, None; **Paul G. FitzGerald**, None; **Robert Getts**, None; **Liliana Werner**, None; **Nick Mamalis**, None; **Mindy George-Weinstein**, None

Program Number: 3636 **Poster Board Number:** B0195
Presentation Time: 3:45 PM–5:30 PM
Cholesterol bilayer domain in eye lens health
Witold K. Subczynski[1], Justyna Widomska[2], Laxman Mainali[1], Marija Raguz[3]. [1]Biophysics, Medical College on Wisconsin, Milwaukee, WI; [2]Biophysics, Medical University of Lublin, Lublin, Poland; [3]Medical Physics and Biophysics, University of Split, Split, Croatia.
Purpose: The most unique biochemical characteristic of the eye lens fiber cell plasma membrane is its extremely high cholesterol content. The cholesterol/phospholipid molar ratio of the human fiber cell membrane ranges from 1 to 2 in the cortex and can be as high as 3 to 4 in the lens nucleus. The need for such a high cholesterol content in the lens is still unclear. It is evident, however, that the disturbance of cholesterol homeostasis may result in damages associated with cataracts.
Methods: Results presented here were obtained using electron paramagnetic resonance spin-labeling methods to discriminate cholesterol bilayer domains (CBDs), and differential scanning calorimetry to discriminate cholesterol crystals. Investigated lipid bilayer membranes were prepared from commercially available lipids and total lipids extracted from clear and cataractous human lens cortexes and nuclei as well as from the eyes of different animals. In key experiments, membranes were prepared using a rapid solvent exchange method.
Results: The extremely high (saturating) content of cholesterol in the fiber-cell membrane keeps the bulk physical properties of the lipid-bilayer portion of the membrane consistent and independent of changes in the phospholipid composition. The CBD provides buffering capacity for cholesterol concentration in the surrounding phospholipid bilayer, keeping it at a constant level of saturation. The high cholesterol content in fiber-cell plasma membranes ensures that the lipid bilayer portion of these membranes forms the high hydrophobic barrier for permeation of polar molecules. The lipid bilayer portion of lens membranes, with its unique lipid composition and structure, forms significant barriers to oxygen transport into the lens interior.
Conclusions: The presence of CBDs seems to play an integral role in the regulation of cholesterol-dependent processes in fiber cell membranes and in the maintenance of fiber cell membrane homeostasis. Obtained results suggest that the high cholesterol content, formation of CBDs, and formation of cholesterol crystals should not be considered as major predispositions for the development of age-related cataracts.
Commercial Relationships: Witold K. Subczynski, None; **Justyna Widomska**, None; **Laxman Mainali**, None; **Marija Raguz**, None
Support: NIH Grant EY015526, EY001931, EB001980

Program Number: 3637 **Poster Board Number:** B0196
Presentation Time: 3:45 PM–5:30 PM
Amounts of phospholipids and cholesterol in lipid domains formed in intact lens membranes: Methodology development and its application to studies of porcine lens membranes
Laxman Mainali[1], Marija Raguz[2], William J. O'Brien[3], Witold K. Subczynski[1]. [1]Biophysics, Medical College of Wisconsin, Milwaukee, WI; [2]Medical Physics and Biophysics, University of Split, Split, Croatia; [3]Ophthalmology, Medical College of Wisconsin, Milwaukee, WI.
Purpose: The proposed research aimed to obtain detailed information about the distribution of phospholipids and cholesterol between lipid domains in intact fiber-cell plasma membranes isolated from eye lenses. This information will help to better describe and understand the organizational changes in human lens fiber-cell membranes that occur with age and during cataract development.
Methods: A new method, based on saturation-recovery (SR) electron paramagnetic resonance (EPR) spin-labeling, has been developed that allows quantitative evaluation of the amount of phospholipids and cholesterol in lipid domains of intact fiber-cell plasma membranes isolated from cortical and nuclear regions of eye lenses. This new method will complement the existing method, which is based on an analysis of conventional EPR spectra of spin labels. Both methods allowed more detailed information about the distribution of phospholipids and cholesterol between domains to be obtained.
Results: Results confirmed that, in nuclear porcine membranes, the amounts of phospholipids and cholesterol in trapped lipid domains created due to the presence of membrane proteins were greater than those in cortical membranes. The sample-to-sample preparation/technique-related changes were evaluated for cortical and nuclear lens membranes prepared from single porcine eyes. This analysis allowed the differences of mean values, which were statistically significant with $p \leq 0.05$, to be determined.
Conclusions: Human lenses differ not only because of age, but also because of the varying health histories of the donors. Thus, single donor and/or single eye experiments are critical. It is necessary to separate health-history-related changes from preparation/technique-related changes, even for a single eye. The statistically significant differences defined for porcine lenses will be used to compare the amounts of lipids in domains in human lens membranes prepared from the eyes of single donors and from single eyes. Greater separations will indicate that differences were statistically significant (with $p \leq 0.05$) and that they are due to sources other than preparation/techniques.
Commercial Relationships: Laxman Mainali, None; **Marija Raguz**, None; **William J. O'Brien**, None; **Witold K. Subczynski**, None
Support: NIH Grant EY015526, EY001931, EB001980

Program Number: 3638 **Poster Board Number:** B0197
Presentation Time: 3:45 PM–5:30 PM
Evidence of Transient Receptor Potential Vanilloid 1 (TRPV1) Channel-mediated Signaling in Lens Epithelium
Amritlal Mandal[1], Mohammad Shahidullah[1, 2], Nicholas A. Delamere[1, 2]. [1]Physiology, College of Medicine, Univ of Arizona, Tucson, AZ; [2]Department of Ophthalmology & Vision Science, University of Arizona, Tucson, AZ.
Purpose: Recently we determined that the Transient Receptor Potential Vanilloid 4 ion channel (TRPV4) in the lens epithelium plays crucial signaling role in a pathway that regulates lens Na,K-ATPase-mediated transport activity. Here we show expression of different TRP channel, TRPV1, linked to signaling pathways in the lens epithelium.
Methods: Western blot analysis was used to probe expression of TRPV1 protein in porcine lens epithelium as well as ERK1/2 and p38 activation. Cytoplasmic calcium was measured by ratiometric imaging of cultured lens epithelial cells loaded with Fura-2 AM. Results (mean ± SE) were analyzed by t-test or 1-way ANOVA ($p < 0.05$ considered significant).
Results: TRPV1 protein was detected in the lens epithelium, equatorial and cortical fibers as well as in cultured lens epithelium. A transient increase in cytoplasmic calcium (Control 157.69±5.79 nM, vs capsaicin 233.45±21.69 nM, p=0.028, n=3) was observed when cultured lens epithelial cells were exposed to a selective TRPV1 agonist, capsaicin (100 nM). Intact lenses exposed to 100 nM capsaicin responded with a rapid (<2 min), robust transient increase in ERK1/2 phosphorylation (38.92±7.56 fold, p=0.0011, n=3) and

p38 MAPK phosphorylation (5.93±0.56 fold, p<0.0001, n=3) in the epithelium. Lenses challenged with hyperosmotic solution (350 mOsm) also showed transient activation of ERK1/2 (8.54±2.76 fold, p=0.012, n=3) and p38 (4.74±0.83 fold, p=0.003, n=3) in the epithelium. Interestingly, the magnitude of ERK1/2 and p38 MAPK activation was significantly reduced by 49.11±3.98% (p=0.005, n=3) and by 34.73±1.24% (p=0.0001, n=3), respectively when lenses were exposed to hyperosmotic solution in the presence of a selective TRPV1 antagonist, A889425 (1.0 μM).
Conclusions: Lens cells express functional TRPV1 channels and their stimulation causes ERK1/2 and p38 MAPK activation in lens epithelium. The evidence points to TRPV1 stimulation in response to hyperosmotic solution, or the shrinkage it causes.
Commercial Relationships: Amritlal Mandal, None; **Mohammad Shahidullah**, None; **Nicholas A. Delamere**, None
Support: NIH Grant EY009532

Program Number: 3639 **Poster Board Number:** B0198
Presentation Time: 3:45 PM–5:30 PM
Maintaining Epithelial Cell Viability in Whole Lens Cultures *ex vivo*
Bharat Kumar, Matthew A. Reilly. Biomedical Engineering, The Ohio State University, Columbus, OH.
Purpose: Lens explants have previously been used to study the behavior of lens epithelial cell (LEC) in vitro but show altered LEC morphology. We therefore evaluated the viability of LECs when culturing whole lenses in vitro in the presence and absence of vitreous humor.
Methods: Pairs of freshly enucleated porcine eyes were dissected and the crystalline lens was removed. One lens from each pair was cultured in growth media for 24 hours while the other was immediately dissected. Lenses were cultured in M199 media which, in some cases, was augmented with vitreous humour in a 1:4 ratio. LEC viability was quantified using a hemocytometer after removing the fiber cell bundle and trypsinizing the anterior lens capsule. The viable cell counts from the cultured lenses were normalized to data from the fresh lenses of each pair.
Results: The normalized viable cell population for lenses cultured in the vitreous enhanced
media was 1.07. The normalized population for the control media was 0.87. Preliminary results indicate that the vitreous-enhanced media played a role in maintaining lens epithelial cell viability (Figure 1).
Conclusions: Viable cell populations in lenses cultured in enhanced media remained consistent after the culture period in contrast to the rapid decrease in lenses cultured in the control media. This information will enable longer-term cultures to determine the influence of additional factors in LEC viability and proliferation.

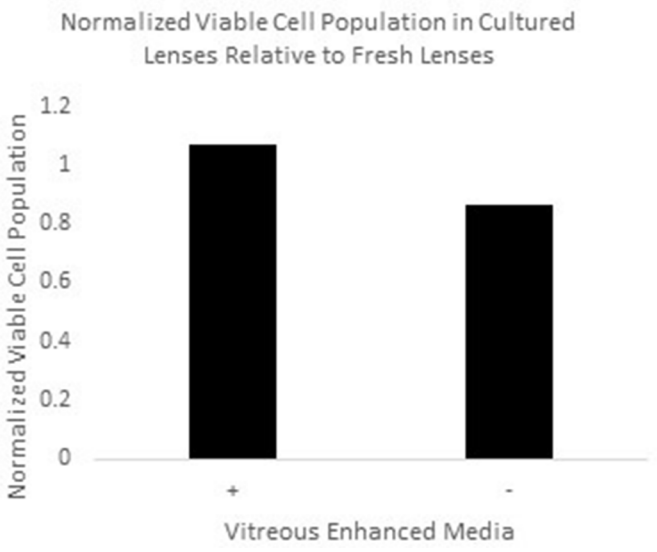


Figure 1. Normalized viable cell populations in lenses cultured in enhanced (left) and control (right) media relative to the viable cell population in fresh lenses
Commercial Relationships: Bharat Kumar, None; **Matthew A. Reilly**, None

Program Number: 3640 **Poster Board Number:** B0199
Presentation Time: 3:45 PM–5:30 PM
Src Family Tyrosine Kinase Signaling in Lens Epithelium is Linked to Calcium-activated Adenylate Cyclase
Mohammad Shahidullah[1, 2], Amritlal Mandal[1], Nicholas A. Delamere[1, 2]. [1]Physiology, Univ of Arizona, College of Medicine, Tucson, AZ; [2]Ophthalmology and Visual Sciences, University of Arizona, Tucson, AZ.
Purpose: Previously we showed exposure of intact lenses to hyposmotic solution causes a Src Family Kinase (SFK)-dependent increase in Na,K-ATPase activity in the epithelium. Here we examined the mechanism that links osmotic stress to SFK activation.
Methods: Intact porcine lenses were exposed to hyposmotic solution (200 mOsm) for 2-10 min and then the epithelium was isolated and used for measurement of cAMP, Na,K-ATPase activity and SFK phosphorylation. SFK phosphorylation (activation) was studied by Western blot analysis, cAMP by RIA and Na,K-ATPase activity as ouabain-sensitive ATP hydrolysis in cell homogenates. Results (mean ± SE) were analyzed by t-test or 1-way ANOVA (p <0.05 considered significant).
Results: When lenses were exposed to hyposmotic solution (2-5 min), SFK phosphorylation in the epithelium was increased (1.45± 0.07 fold, p=0.0001, n=3). The SFK phosphorylation response was not altered in lenses exposed to hyposmotic solution in the presence of a soluble guanylate cyclase inhibitor ODQ (10 μM) that inhibits the Ca^{2+}-NOS-cGMP-PKG pathway. In contrast, the hypotonicity-induced SFK phosphorylation was abolished by a cytoplasmic Ca^{2+} chelator, BAPTA-AM (10μM) (Con 1.0±0., hypo 1.59±0.17 vs BAPTA+hypo 0.66±0.15, p=0.0009, n=3), by a TRPV4 antagonist, HC 067047 (10 μM) (Con 1.0±0, hypo 1.47±0.10 vs HC+Hypo 1.12±0.08 p=0.0325, n=3), and by a selective protein kinase A inhibitor, H89 (10 μM) (Con 1.0±0, Hypo 1.45±0.15 vs H89+hypo 1.08±0.02, p=0.02, n=3). The data suggest hypotonicity-induced SFK activation may follow TRPV4-mediated Ca^{2+} entry and subsequent activation of a Ca-dependent AC. Consistent with this notion, hyposmotic solution caused a rapid increase of cAMP in

the lens epithelium at 2 min (6.17±0.42 vs 8.48±0.6, p=0.014, n=8) and at 5 min 5.54 ±0.34 vs 12.50±2.20 (p=0.011, n=6) pmol cAMP/mg protein. Moreover, BAPTA-AM suppressed the hypotonicity-induced Na,K-ATPase activity increase (Control 48.32±3.05, hypo 88.6±6.64 vs BAPTA-treated 44.33±3.34, p=0.0011, n=4) nmoles ATP hydrolyzed/mg protein/30 min. Ca-dependent adenylate cyclase 3 (ADCY3) and 8 (ADCY8) are expressed in the lens epithelium.
Conclusions: The findings indicate SFK activation in the epithelium of lenses exposed to hyposmotic solution is linked to stimulation of a Ca-dependent adenylate cyclase, production of cAMP and activation of protein kinase A. The mechanism depends on Ca^{2+} entry via TRPV4 channels.
Commercial Relationships: Mohammad Shahidullah, None; **Amritlal Mandal**, None; **Nicholas A. Delamere**, None
Support: NIH Grant EY009532

Program Number: 3641 **Poster Board Number:** B0200
Presentation Time: 3:45 PM–5:30 PM
Effects of Combined Circadian Rhythm Disruption and Alcohol on Murine Lens Structure
Kristin J. Al-Ghoul[1], Robin M. Voigt[2], Christopher B. Forsyth[2], Ali Keshavarzian[2]. [1]Anatomy & Cell Biology, Rush University Medical Center, Chicago, IL; [2]Internal Medicine, Section of Gastroenterology, Rush University Medical Center, Chicago, IL.
Purpose: It is generally accepted that cataract formation is a multifactorial process, and two factors that are associated with the development of lens opacities are alcohol abuse and circadian rhythm disruption. The present study was conducted to determine if the morphology and organization of lens fiber cells is altered after circadian rhythm disruption in conjunction with alcohol exposure.
Methods: The study utilized male C57BL/6J mice (n=58) housed in either constant light-dark (LD) 12:12 conditions or with weekly phase shifts in the LD 12:12 cycle for 3 months prior to the start of a 10-week period where the mice were fed either an alcohol-containing Nanji diet or an iso-caloric control diet, in which the alcohol calories are replaced with dextrose. Chow-fed mice were also included as a naive control group. Thus there were 5 experimental groups: 1) non-shifted, alcohol-fed, 2) non-shifted, control-fed, 3) shifted, alcohol-fed, 4) shifted, control-fed, 5) chow-fed. Animals were sacrificed at six ZT (Zeitgeber Time) points: 0, 4, 8, 12, 16, 20. Lenses were removed, then fixed, embedded, sectioned and stained for microscopic examination.
Results: Lenses from shifted, alcohol-fed mice had structural disruptions in superficial anterior, posterior and equatorial regions. Non-shifted, alcohol-fed murine lenses were affected primarily in the anterior region, with only occasional alterations in the posterior region and no structural changes in the equatorial region. As expected, both shifted and non-shifted dextrose fed animals had disruptions of the superficial fibers and epithelium, which were consistent with 'sugar cataracts.' No marked structural differences were noted between ZT groups within a given treatment group. Lenses from chow-fed mice lacked the structural changes seen in the 4 experimental groups.
Conclusions: Animals subjected to the double-hit of circadian rhythm disruption and alcohol consumption showed the most extensive lenticular damage as compared to alcohol-only treated and naïve control animals. This indicates that circadian rhythm disruption may exacerbate the effects of alcohol on the maintenance of lens fiber structure. We speculate that systemic inflammation could be an underlying mechanism linking alcohol, circadian rhythm disruption and cataract formation.
Commercial Relationships: Kristin J. Al-Ghoul, None; **Robin M. Voigt**, None; **Christopher B. Forsyth**, None; **Ali Keshavarzian**, None
Support: Mary Lou Bell McGrew Fund, Rush University, Chicago, IL (KJA); NIH Grant AA020216 (AK)

Program Number: 3642 **Poster Board Number:** B0201
Presentation Time: 3:45 PM–5:30 PM
Crystalline lens change peculiar to diabetes
Hiroshi Sasaki, Natsuko Hatsusaka, Hisanori Miyashita, Naoko Shibata, Yusuke Seki, Teppei Shibata, Hiromi Osada, Hidetoshi Ishida, Masami Kojima, Eri Kubo. Department of Ophthalmology, Kanazawa Medical University, Kahoku-gun, Japan.
Purpose: We investigated the relationship among diabetes mellitus (DM), diabetic retinopathy (DMR) and crystalline lens changes by epidemiological surveys conducted in Japan, China, Taiwan, Iceland and Tanzania.
Methods: The epidemiological investigation included 1778 participants aged over 50 (mean: 61.5±9.5) years, comprising 115 Japanese residents of Wajima city, 1054 Chinese residents of Sanya and Taiyuan cities, and 609 Tanzanian residents of Mkuranga district. DM was confirmed by self-report. Cataract and DMR were diagnosed by one experienced ophthalmologist (HS) using a slit lamp microscope. Cataracts were grouped into 5 types: cortical (COR), nuclear (NUC), posterior subcapsular cataract (PSC), Retrodots (RD) and Waterclefts (WC). WC with vacuoles were grouped as VWC. COR and WC comprised 2 types: central (CEN+) within 3 mm diameter of the pupil center and peripheral (CEN-) outside this area. We used the Statistical Package for Social Science (SPSS) for logistic regression analysis, taking age, gender, smoker/non-smoker, and axial length and total UV exposure dose as objective variables to examine the risks of 5 types of cataracts with/without DM and DMR.
Results: Regarding DM, the odds ratios were: COR (CEN-), 1.67 (1.12-2.48, 95% CI) ($p<0.05$); NUC, 0.62 (0.38-1.02) ($p=0.062$); PSC, 2.48 (1.36-4.52) ($p<0.01$); RD, 1.54 (1.08-2.20) ($p<0.05$); WC (CEN+), 0.32 (0.12-0.85) ($p<0.05$); and VWC (CEN-), 3.60 (2.10 - 6.15) ($p<0.001$). Regarding DMR, the odds ratios were: RD, 5.6 (2.1-14.8); and VWC (CEN-), 14.0 (3.6-54.9) ($p<0.01$). In the subjects with and without VWC (CEN-), prevalence of DM was 23.1% and 4.1%, and that of DMR, 12.8% and 1.3%, which were significantly higher in subjects with VWC (CEN-) ($p<0.001$).
Conclusions: The risks of RD and VWC (CEN-) in addition to cortical and posterior subcapsular cataract are high in patients with DM. Accordingly, these lens lesions may serve as a diagnostic tool in treatment of DM.
Commercial Relationships: Hiroshi Sasaki, None; **Natsuko Hatsusaka**; **Hisanori Miyashita**, None; **Naoko Shibata**, None; **Yusuke Seki**, None; **Teppei Shibata**, None; **Hiromi Osada**, None; **Hidetoshi Ishida**, None; **Masami Kojima**, None; **Eri Kubo**, None

Program Number: 3643 **Poster Board Number:** B0202
Presentation Time: 3:45 PM–5:30 PM
Pax6 Sumoylation Is Enhanced In Cataract Lenses
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Purpose: Our recent study revealed that SUMO1-mediated sumoylation is an indispensable step towards activation of p32 Pax-6, a master regulator of eye and brain development (Yan et al. 2010. PNAS). However, whether Pax6 sumoylation is altered during lens

pathogenesis remains unknown. In this study, we have examined Pax6 sumoylation status from patient cataract lenses.
Methods: Immunocytochemistry and Western blot analysis were used to detect Pax6 sumoylation status in the cataract lenses from patents.
Results: Compared with the normal transparent lenses, the cataract lenses from different patients have strong 32 kd Pax6 expression. The 48 kd Pax6 is also present. In contrast, the 46 kd Pax6 is largely sumoylated, In addition, a partial 32 kd Pax6 is also sumoylated.
Conclusions: Sumoylation pattern of Pax6 is changed during lens pathogenesis. [Supported by the grant (No. 81570824) from the National Natural Science Foundation of China, Research Prevent Blindness to UNMC, and the Fundamental Funds of the State Key Laboratory of Ophthalmology in Zhongshan Ophthalmic Center, and the Graduate Scholarship from Sun Yat-sen University]
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Presentation Time: 3:45 PM–5:30 PM
Expression of SUMO Isoforms in Different Compartments of Mouse Eye
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Purpose: Our recent studies have shown that SUMO1 and SUMO2/3 have distinct functions in regulating lens differentiation (Gong et al. 2014 PNAS). To further explore the functions of sumoylation in othee tissues of the vertebrate eye, here we have characterized the expression patterns of different SUMO isoforms in these tissues.
Methods: QRT-PCR, Immunocytochemistry and Western blot analysis were used for the detection of SUMO isoform expression patterns.
Results: Compared with SUMO2/3, SUMO1 is highly expressed in the cornea, retina and lens epithelial cells. The number of proteins conjugated by SUMO1 is: retina>lens epithelial cells>cornea>Lens fiber cells. Unconjugated SUMO isoforms are hardly detected in C57 mice.
Conclusions: SUMO isoforms differentially conjugated to various proteins in retina, lens and cornea. Sumoylation may play an important role in different tissues of the vertebrate eye. [Supported by the grant (No. 81570824) from the National Natural Science Foundation of China, Research Prevent Blindness to UNMC, and the Fundamental Funds of the State Key Laboratory of Ophthalmology of Zhongshan Ophthalmic Center, and the Graduate Scholarship from Sun Yat-sen University]
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Expression of the Sumoylation Enzymes in the Ocular Tissues of Mice
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Purpose: The mammalian small ubiquitin-like modifiers (SUMOs) are actively involved in regulating differentiation of different cell types. In ocular tissues, sumoylation helps to determine the differentiation of cone verse rod photoreceptors. Our recent study revealed that SUMO1 and SUMO2/3 display distinct functions in regulating lens differentiation (Gong et al., 2014. PNAS). Here, we determined the expression patterns of different sumoylation enzymes in various ocular tissues
Methods: QRT-PCR, Immunocytochemistry and Western blot analysis were used for analyse the expression patterns of both sumoylation ligases and desumoylation enzymes from 5-week C57 mice.
Results: Ligase 1 (SAE2), 2(Ubc9) and 3 (Pias1) are all strongly expressed in the cornea, retina and lens epithelial cells. Similar results are obtained for the desumoylation enzyme (SENP1). Among the 3 compartments, these sumoylation and desumoylation enzymes are much highly expressed in retina, intermediately expressed in the cornea but lower in lens epithelial cells and the lowest in lens fiber cells.
Conclusions: Sumoylation may play an important role in all 3 compartments of mouse eye to maintain the normal visual physiology [Supported by the grant (No. 81570824) from the National Natural Science Foundation of China, Research Prevent Blindness to UNMC, and the Fundamental Funds of the State Key Laboratory of Ophthalmology of Zhongshan Ophthalmic Center in Sun Yat-sen University]
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