335 Cellular Metabolism of the Retina and RPE
Tuesday, May 09, 2017 11:00 AM–12:45 PM
Exhibit/Poster Hall Poster Session
Program #/Board # Range: 3006–3028/A0001–A0023
Organizing Section: Retinal Cell Biology

Program Number: 3006 **Poster Board Number:** A0001
Presentation Time: 11:00 AM–12:45 PM
Glucocorticoids and survival of retinal pigment epithelial cells (ARPE-19) in vitro
Melisa Daniela D. Marquioni Ramella, Pablo S. Tate, Tomas P. Bachor, Mariela C. Marazita, Angela M. Suburo. Facultad de Ciencias Biomédicas, IIMT, Universidad Austral-CONICET, Presidente Derqui, Pilar, Argentina.
Purpose: Glucocorticoids (GC) are required for photoreceptor survival, and are useful for retinal protection. However, little information is available about the role of GC in the retinal pigment epithelium (RPE). Therefore we tested ARPE-19 cells, a human RPE cell line, to find out whether survival and proliferation could be modified by dexamethasone (DEX), a specific agonist of GC receptor α (GRα), or mifepristone (MFP), a GC antagonist and a possible agonist of GC receptor β (GRβ).
Methods: ARPE-19 cells were cultured for 48 h in DMEM/F12 with 10% fetal calf serum and antibiotics with 5% CO2 in air at 37°C. Cultures were then treated with DEX (0.08, 0.32, 1.28 mM) and/or MFP (1, 10 μM, both solubilized in ethanol). Controls were made in medium with or without ethanol. After 24 h, cultures were stained with acridine orange and ethidium bromide (AO/EB) for detection of apoptotic and necrotic cells. Other cultures were incubated in 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) during 4 h at 37°C to measure cell viability. The expression of GRβ was assayed in control cultures using Western blots or qPCR. Balb-c mice eye sections were immunostained with GRβ antibody.
Results: The AO/EB procedure showed that 0.08 mM DEX cultures displayed 3% of necrotic/apoptotic cell nuclei, whereas 1.28 mM DEX exhibited 37% of cells with necrotic/apoptotic signs. MFP (1 and 10 μM) induced less than 5% of necrotic/apoptotic cell nuclei. Cell viability after 0.08 mM DEX was not different from controls, but it decreased after 0.32 or 1.28 mM DEX ($p < 0.001$). By contrast, MFP did not modify cell viability. Combination of 0.08 mM DEX with 1 or 10 μM MFP did not induce viability changes. Exposure to 1.28 mM DEX and MFP (1 or 10 μM) reduced viability in the same proportion as 1.28 mM DEX alone.
Both Western blots and qPCR demonstrated high expression of GRβ in ARPE-19 cells. Moreover, the mouse RPE showed a much higher GRβ immunoreactivity than the neural retina.
Conclusions: Assays for cell death and cell viability demonstrated DEX cytotoxicity for ARPE-19 cells. These effects were not antagonized by MFP, suggesting that they would not be mediated by GRα receptors. Alternatively, the availability of MFP could be decreased by binding to GRβ. The selective localization of this receptor in RPE cells indicates that further studies should be carried out to understand its role in retinal physiology and protection.
Commercial Relationships: Melisa Daniela D. Marquioni Ramella, None; **Pablo S. Tate**, None; **Tomas P. Bachor**, None; **Mariela C. Marazita**, None; **Angela M. Suburo**, None
Support: PICT-2013-3200

Program Number: 3007 **Poster Board Number:** A0002
Presentation Time: 11:00 AM–12:45 PM
Exploration of the resistance's mechanisms to oncogenesis induced by APC mutation in retinal pigment epithelium cells
REGENT Florian[1], Alexandra Plancheron[1, 2], Karim Ben M'Barek[1], Walter Habeler[1, 2], Laure Chatrousse[1, 2], Pascal Fragner[1, 2], Yacine Laâbi[1, 2], Christelle Monville[1]. [1]I-Stem, INSERM UEVE UMR861, AFM, Corbeil-Essones, France; [2]I-Stem, CECS AFM, Corbeil-Essones, France.
Purpose: Familial Adenomatous Polyposis (FAP) is characterized by colonic adenomas but also by various extra-intestinal manifestations. Among these manifestations, pigmented ocular fundus lesion (POFL) is the most common. Clinically these lesions are similar to congenital hypertrophy of the retinal pigment epithelium (CHRPE) but postmortem studies in FAP patients demonstrated RPE hyperplasia and RPE hamartomata's growth in addition to CHRPE. Although the RPE cells seem sensitive to oncogenesis induce by APC, these lesions never derived into Adenocarcinoma. It suggests that APC mutation in RPE cells activates first an oncogenic process and, in a second time, a mechanism of tumor suppression that stops lesion progression. We hypothesize that APC mutation results into oncogene induced senescence in RPE cells and mechanisms underlying this process are under investigation.
Methods: WT (N=2 lines) and APC mutant (N=2 lines) human embryonic stem cells (hESCs) are differentiated into RPE cells to establish the cellular model. In addition, an isogenic APC mutant hESC line has been generated using the CrispR/Cas9 technology. These cells are then compared to identify pathological phenotypes and markers. Cell-cell junctions, epithelial organization, proliferation and senescence markers were explored by immunostaining and immunoblot at day 7, 14, 21 and 35. Moreover pigmentation levels of WT and mutant cells were compared by macroscopic observation and by the quantification of the melanin and the expression of proteins involved in the melanin synthesis pathway.
Results: Preliminary results indicate that APC mutated RPE cells express high level of KI67 at day 21 compare to WT cells, suggesting an over-proliferation and a loss of inhibition contact. APC Mutated RPE cells also exhibit local epithelial disorganization with the loss of the monolayer aspect of the epithelium. Moreover APC mutation induced a hyperpigmentation of the RPE associated with an overexpression of MITF and Tyrosinase.
Conclusions: hESCs derived RPE cells harboring APC mutation recapitulate the main phenotypes associated to POFLs observed in FAP patients. Consequently these cells represent an interesting model to study the mechanisms that protect RPE against oncogenesis induced by APC mutation. We will now determine if APC mutation induces specific senescence in long term cultures.
Commercial Relationships: REGENT Florian, None; **Alexandra Plancheron**, None; **Karim Ben M'Barek**, None; **Walter Habeler**, None; **Laure Chatrousse**, None; **Pascal Fragner**, None; **Yacine Laâbi**, None; **Christelle Monville**, None

Program Number: 3008 **Poster Board Number:** A0003
Presentation Time: 11:00 AM–12:45 PM
Stillbene compounds induce dynamic autophagy and decrease protein aggregation during proteasome inhibition
Kai Kaarniranta[1, 2], Johanna Viiri[1], Mika Reinisalo[3], Ali Koskela[1]. [1]Department of Ophthalmology, University of Eastern Finland, Kuopio, Finland; [2]Department of Ophthalmology, Kuopio University Hospital, Kuopio, Finland; [3]School of Pharmacy, University of Eastern Finland, Kuopio, Finland.
Purpose: Decreased autophagic and proteasomal cleansing has been documented in the degeneration of retinal pigment epithelial

(RPE) cells and the pathology of age-related macular degeneration. Recently, polyphenolic compounds has been actively studied in the prevention of age-related diseases. We examined effects of resveratrol and another stilbene compound, pinosylvin, on the regulation of autophagy and cytoprotection in ARPE-19 and mouse primary RPE cells under proteasome inhibition.
Methods: Protein aggregation was caused with proteasome inhibitor MG-132 (1 microM). Autophagy was inhibited with bafilomycin A1 (50 nM). Resveratrol (25 microM) or pinosylvin (15 microM) were used solely and together with proteasome inhibition up to 48 hours. A stress marker Hsp70 (Heat Shock Protein 70) and autophagy regulators p62 (SQSTM1) and LC3 (microtubule-associated protein 1A/1B-light chain 3) were analyzed by Western blotting (WB). Tandem fluorescently tagged LC3 (GFP-mCherry-LC3) transfection was used to study autophagy flux in fluorescence microscopy for ARPE-19 cells.
Results: Inhibition of proteasomes with MG-132 upregulated Hsp70, autophagy markers p62 and LC3 detected in WB. Simultaneous treatment with MG-132 and resveratrol or pinosylvin decreased amount of Hsp70, p62 and LC3. However, the levels of p62 and especially LC3-I/LC3-II fluctuated during the monitored time period revealing a dynamic autophagy process. Resveratrol and pinosylvin provided similar effects on the decreased protein aggregation under proteasome inhibition. Fluorescence microscopy showed increased autophagy flux with GFP-mCherry-LC3. Resveratrol or pinosylvin with autophagy inhibition resulted accumulation of p62 and LC3-II.
Conclusions: Stillbenes are potent inducer of autophagy and prevent cell damage during proteasome inhibition in RPE cells.
Commercial Relationships: Kai Kaarniranta, None; **Johanna Viiri**, None; **Mika Reinisalo**, None; **Ali Koskela**, None

Program Number: 3009 **Poster Board Number:** A0004
Presentation Time: 11:00 AM–12:45 PM
Epithelial-Mesenchymal Transition of Retinal Pigment Epithelium from Different Sources *in Vitro*
Bo Lu[1], Wei Hu[1], Xinxin Zhang[1], Na Yang[1], Neil Bressler[2], Jun Kong[1, 2]. [1]Ophthalmology, The Fourth Affiliated Hospital of China Medical University, Shenyang, China; [2]Retina Division, Wilmer Eye Institute, JHH, Baltimore, MD.
Purpose: To investigate features associated with initiation of mesenchymal changes when retinal pigment epithelium (RPE) cells are subcultivated serially *in vitro* and to compare how to change peculiarities of fetal RPE and RPE differentiated from human embryonic stem cells (hESCs-RPE) after they experience epithelial-mesenchymal transition (EMT).
Methods: fRPE and hESC-RPE cells are respectively subcultured *in vitro and* passaged by trypsin-EDTA in 20min or 10min. The changes in cell configuration are observed by inverted phase contrast microscope and hematoxylin-eosin staining. Real time quantification PCR (qPCR) western blot and immunofluorescent assays were employed to analyze changes in biomarkers (epithelial markers: RPE65, E-cadherin, ZO-1; mesenchymal markers are: alpha smooth muscle actin(α-SMA), N-cadherin, Fibronectin) of fetal RPE and hESCs9-RPE.
Results: 1. Normal fRPE and hESC-RPE cells presented a cobblestone appearance with pigment surrounding the nuclei. However, when RPE cells had abundant tonofilament, pigment sharply decreased or disappeared, which suggested mesenchymal changes. The proliferating ability of cells reduced when continuously passaged *in vitro*, especially in EMT transdifferentiated cells. 2. hESCs-RPE and fRPE passed with a concentration of $6x10^5$ cells/ml and complete dissociation during passagingmight show EMT characteristics and express mesenchymal markers as early as in the fourth passage, while RPE cells passed with a concentration of $3x10^5$ cells/ml or insufficient trypsinization time might transdifferentiate as early as in the first passage. 3. Results of qRT-PCR analysis showed increased a-SMA, N-cadherin, fibronectin mRNA expression and decreased RPE65, E-cadherin and ZO-1 mRNA expression ($p < 0.05$) after RPE experienced EMT. Western blot and immunoblotting confirmed the increased expression of EMT markers and decreased expression of epithelial markers at protein level. However, hESCs-RPE behaved similarly with EMT changes except E-cadherin expression increased.
Conclusions: These findings suggest that EMT change can occur when RPE from aborted fetuses and human embryonic stem cells are cultured *in vitro*. There are also differences in protein expression between fetal RPE and hESCs9-RPE. These data also suggest in future clinical RPE transplantation, one should consider the condition of RPE cells as well as their different sources.
Commercial Relationships: Bo Lu, None; **Wei Hu**, None; **Xinxin Zhang**, None; **Na Yang**, None; **Neil Bressler**, None; **Jun Kong**, None
Support: The Science & Technology Program of Shenyang Department of Science and Techmology (NO. F14-208-6-00)

Program Number: 3010 **Poster Board Number:** A0005
Presentation Time: 11:00 AM–12:45 PM
The Role of Oxidative Stress Pathway in RPE Epithelial to Mesenchymal Transition
Karla Y. Barbosa[1], Justin Chang[1], Madhu Lal[2], Kapil Bharti[1]. [1]NEI, NIH, Bethesda, MD; [2]National Center for Advancing Translational Sciences, NIH, Bethesda, MD.
Purpose: Purpose: Retinal injury often triggers retinal pigment epithelial (RPE) cells to undergo epithelial to mesenchymal transition (EMT) leading to a condition called proliferative vitreoretinopathy (PVR). PVR causes fibrosis and can cause retinal detachment leading to severe vision loss. The EMT process is characterized by the switch of epithelial phenotype of the RPE into fibroblast phenotype. The aim of this study is to determine key molecular players involved in EMT of the RPE with the goal to discover potential therapeutic targets.
Methods: Methods: We performed a siRNA screen to identify candidate genes and pathways required to maintain epithelial phenotype of iPSC-RPE. A reporter induced pluripotent stem (iPS) cell line that expresses GFP when differentiated into RPE cells was used for the siRNA screen. An *in vitro* RPE injury model was developed to validate the identified pathways. Human iPS cell derived RPE cultured in monolayer were subject to mechanical injury to induce EMT. Gene expression was confirmed by immunocytochemistry and qRT-PCR. Various signaling pathways were manipulated using pharmacological and genetic inhibitors.
Results: Results: siRNA screen identified several previously known pathways including tight junction and MAP kinase pathways critical for regulating EMT process in RPE cells. In addition, this screen identified oxidative stress as a potentially novel pathway in regulating epithelial phenotype of iPSC-RPE. Results from siRNA screen were validated in our *in vitro* RPE injury model using immunohistochemistry and gene expression for key oxidative stress & EMT pathway components (FOXO, TGF-beta, BMP1, ZEB1). FOXO, a key oxidative stress response transcription factor was localized to the nucleus in injured RPE cells during EMT. This was co-incidental with increased levels of ROS in the injured RPE cells undergoing EMT.
Conclusions: Conclusions: Overall these findings suggest that oxidative stress plays a role in the RPE-EMT and PVR response. These findings contribute to the knowledge about the multiple

signaling pathways are involved in EMT and provide the possibility of identifying potential therapeutic targets for PVR.
Commercial Relationships: Karla Y. Barbosa, None; **Justin Chang**, None; **Madhu Lal**, None; **Kapil Bharti**, None

Program Number: 3011 **Poster Board Number:** A0006
Presentation Time: 11:00 AM–12:45 PM
Circadian regulation of mitochondrial dynamics in retinal photoreceptors
JANET Ya-An CHANG, Laurie Sheldon, Michael L. Ko, Gladys Y. Ko. Veterinary Integrative Biosciences, Texas A&M University, College Station, TX.
Purpose: We previously demonstrated that the circadian oscillators in avian photoreceptors regulate photoreceptor physiology and metabolism. In this study, we further determined the circadian oscillations of mitochondrial dynamics in cultured 661W cells.
Methods: The 661W cells were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal Bovine serum, 1% glutamax and 1% penicillin-streptomycin antibiotics at 37°C in 5% CO2. Incubators were equipped with lights and timers for the circadian entrainment to 12:12 h light-dark (LD) cycles in vitro. After 5 days in cultures under LD entrainment, 661W cells were collected at various Zeitgeber time points for further analyses. After cultured 4 days in LD, some cultured 661W cells were kept in complete darkness (DD) for 2 days, and on the second day of DD, these cells were collected at various circadian time points for further analyses. Chicken embryos from embryonic day 11 were entrained in LD cycles for 7 days in ovo, and retina tissues were collected at various time points for Western blots. Markers for mitochondrial dynamics, mitophagy, and oxidative stress were examined. One-way ANOVA were used for statistical analyses across the time points
Results: : In 661w cells, the protein level of mitofusin 2 (MFN2) was higher during the day and lower at night when cells were under LD, but MFN2 became non-rhythmic in DD. The expression of dynamin related protein 1 (DRP1) was higher at night. The PTEN-induced putative kinase 1 (PINK1), a mitophagy marker, was rhythmic under both LD and DD. Both MFN2 and DRP1 displayed diurnal rhythms in the whole chicken retina, but their rhythmic expressions were antiphase from each other.
Conclusions: Even though 661W cells are a mammalian immortal cone-derived cell-line, our results showed that 661W cells can be entrained in cyclic lights. While mitochondrial fission and fusion dynamics is apparent to be light-driven, the mitochondrial response to stress might be under the circadian control in 661W cells.
Commercial Relationships: JANET Ya-An CHANG, None; **Laurie Sheldon**, None; **Michael L. Ko**, None; **Gladys Y. Ko**, None
Support: NIHR21EY023339 to GK, a graduate research grant from Texas A&M University College of Veterinary Medicine to JC.

Program Number: 3012 **Poster Board Number:** A0007
Presentation Time: 11:00 AM–12:45 PM
Response of ARPE-19 cybrids of European and African origin to high glucose and bevacizumab
Sean W. Tsao, Abdul Sami Memon, Sina Abedi, Mohammad Riazi Esfahani, Mohamed Mohamed, Sonali R. Nashine, Marilyn Chwa, Cristina M. Kenney, Baruch D. Kuppermann. Ophthalmology, Gavin Herbert Eye Institute, Irvine, CA.
Purpose: Oxidative stress and mitochondrial dysfunction have been implicated in the pathway to cell death in diabetic retinopathy. Mitochondrial DNA (mtDNA) can be classified into haplogroups that represent different geographic origins of populations. Differences in maternally inherited mtDNA haplogroups can influence susceptibility to various ocular diseases. The influence of mtDNA haplogroups on mitochondrial metabolism in diabetes is not well characterized. The purpose of this study is to describe the differences in bioenergetic profile between mtDNA from European origin (H haplogroup) and African origin (L haplogroup) when exposed to high glucose and bevacizumab (beva) *in vitro.*
Methods: Two groups of cytoplasmic hybrids (cybrids) were created by fusing ARPE-19 cells devoid of mtDNA with platelets from donors of either H or L haplogroups. Both groups of cybrids were exposed to: 1) normal glucose only (NG), 2) high glucose only (HG), 3) normal glucose + beva (NG+B) or 4) high glucose + beva (HG+B). Cells were assayed for levels of reactive oxygen species (ROS) using the fluorescent dye 2', 7'-dichlorodihydrofluorescein diacetate, cell viability (CV) measured by MTT assay and mitochondrial membrane potential ($\Delta\Psi m$) as measured with JC-1 dye kit. Student's t-test was used to compare mean percent change between sample groups, with the NG only samples set at 100% production or function
Results: The L haplogroup showed a reduction in ROS production with groups NG+B (81.7%, P<0.001) and HG+B (66.6%, P<0.001). The H haplogroup also had a reduction in ROS production, NG+B (76.6%, P<0.001) and HG+B (68.8%, P=<0.001). CV was improved in the NG+B (+46.6%, p<0.001) and HG+B (+29.1%, p=0.001) cultures for the L haplogroup. This positive effect on CV was not seen in the H haplogroup. $\Delta\Psi m$ was significantly reduced in the L haplogroup cultures NG+B (-25.0%, p=0.004) and HG+B (-30.0%, p<0.001), as well as the H haplogroup cultures NG+B (-49.4%, p<0.001) and HG+B (-47.9%, p<0.001).
Conclusions: ROS production was reduced by the addition of beva in both H and L haplogroups. Beva rescued CV in the L haplogroup, but not in the H haplogroup. $\Delta\Psi m$ was significantly reduced by the addition of Beva in both H and L haplogroups. The varied responses between the H (European) and L (African) cybrids to beva may reflect the diversity in mitochondrial response to energy metabolism and cellular damage in different racial populations.
Commercial Relationships: Sean W. Tsao, None; **Abdul Sami Memon**, None; **Sina Abedi**, None; **Mohammad Riazi Esfahani**, None; **Mohamed Mohamed**, None; **Sonali R. Nashine**, None; **Marilyn Chwa**, None; **Cristina M. Kenney**, None; **Baruch D. Kuppermann**

Program Number: 3013 **Poster Board Number:** A0008
Presentation Time: 11:00 AM–12:45 PM
Mitochondrial activity in the retina relies on glycolysis independent of substrate availability
Marin Gantner[1], kevin eade[1], Edith Aguilar[2, 1], Mitchell Prins[1], Alec Johnson[1], Martin Friedlander[2, 1]. [1]The Lowy Medical Research Institute, La Jolla, CA; [2]The Scripps Research Institute, La Jolla, CA.
Purpose: The neural retina has some of the highest biosynthetic and ATP demands in the body. To meet these demands the retina uses a large glycolytic flux as well as high levels of mitochondrial oxidative phosphorylation. The retina is unique in that blocking glycolysis impairs mitochondrial function even when adequate substrates are provided. We are therefore investigating the mechanisms underlying the reliance of mitochondrial function on glycolysis.
Methods: To assess glycolytic and mitochondrial activity we monitored the extracellular acidification and oxygen consumption rates in ex vivo mouse retinas, as well as human stem cell derived eye cups using a Seahorse Flux Analyzer. To inhibit different stages of glycolysis, the retina was treated with either 2-deoxyglucose (2DG), a competitive inhibitor of hexokinase, or iodoacetate, an inhibitor of glyceraldehyde 3-phosphate dehydrogenase (GAPDH). To determine which cell types have a similar response, primary Müller glia, retinal pigment epithelium (RPE) and cells derived from mature human eye cups were tested in a similar manner.

Results: Even in the presence of mitochondrial substrates, pyruvate and glutamine, the oxidative capacity of the retina decreases rapidly when exposed to 2DG. This phenomenon is observed both in the ex vivo mouse retina as well as the human eye cups. Treatment with iodoacetate also reduced oxidative phosphorylation, but to a lesser extent than 2DG. Treatment of primary Müller glia and RPE with 2DG does not impair mitochondrial activity. However, cells derived from the human eye cups maintain partial sensitivity to both 2DG and iodoacetate.
Conclusions: The retina has a unique reliance on glucose. In the absence of glycolysis, oxygen consumption in the mitochondria is blocked even when supplied the downstream product of glycolysis (pyruvate). Given that inhibition of GAPDH has a similar (albeit lesser) effect, indicates that it is not glucose signaling alone, but the flux through glycolysis that is responsible for influencing mitochondrial activity. Unlike primary Müller glia and RPE, human eye cups show a similar sensitivity, as do cells derived from the eye cups. These data indicate 1) that human eye cups are a relevant model to study metabolism in the retina and 2) that the phenomenon is not dependent on the tissue being intact or on metabolic dependency between cell types.
Commercial Relationships: Marin Gantner, None; **kevin eade**, None; **Edith Aguilar**, None; **Mitchell Prins**, None; **Alec Johnson**, None; **Martin Friedlander**, None
Support: Lowy Medical Research Institute

Program Number: 3014 **Poster Board Number:** A0009
Presentation Time: 11:00 AM–12:45 PM
PGC-1α is required to maintain RPE metabolism, integrity and function
Mariana Aparecida B. Rosales, Sarah Melissa Jacobo, Jared Iacovelli, Magali Saint-Geniez. Schepens Eye Research Institute, Massachusetts Eye and Ear Infirmary, Department of Ophthalmology, Harvard Medical School, Chelsea, MA.
Purpose: While mitochondrial biogenesis and autophagy dysfunction have been implicated in the pathogenesis of age-related macular degeneration, the molecular regulation of RPE metabolism remains unclear. We previously identified the transcription co-factor, PGC-1α, as an important inducer of RPE oxidative metabolism and anti-oxidant capacity. The aim of this study was to further characterize PGC-1α roles in RPE cells metabolism, integrity and function.
Methods: PGC-1α expression was silenced in ARPE-19 cells by lentivirus-mediated delivery of pGIPZ-encoded shRNAs specific to the human PGC-1α sequence (shPGC-1α). A pGIPZ non-silencing shRNA (shControl) was used as negative control. Transduced cells were positively selected by FACS sorting and confirmed at the mRNA and protein level. ShPGC-1α and shControl cell lines were maturated under serum starvation for up to 21 days. PGC-1α gain of function was performed by adenoviral transduction. Gene expression of PGC-1 isoforms, mitochondrial dynamic and autophagy was analyzed by qPCR. Mitochondrial morphology and redox status were measured by Mitotracker and MitoSox respectively. Cellular organization and autophagy markers were evaluated by IHC. Barrier function was assessed by transepithelial electrical resistance (TEER).
Results: Following 7 days of maturation, severe disorganization of the mitochondrial network associated with increase in toroidal (donut-shaped) mitochondria and increased mitochondrial superoxide production (p=0.006) was observed in shPGC-1α cells. Evaluation of the RPE barrier function revealed that loss of PGC-1α leads to significant decrease in TEER by day 14 (p=0.03). LAMP-1 positive organelles appeared abnormally dilated at 7 days suggesting that PGC-1α also regulates RPE autophagy. This concept was supported by gene expression analysis showing that PGC-1α is upregulated by autophagy-inducing treatments such as amino acid starvation (p=0.05 at 24 hrs), exposure to 1 mM H_2O_2 (p=0.04 at 6 and 12 hrs) and to 60 nM tunicamycin (p=0.004 at 24 hrs). Gain of function experiments confirmed that PGC-1α induces the expression of autophagosomes biogenesis associated genes such as *WIPI1*, *MAP1LC3B* (LC3) and *ATG4D*.
Conclusions: Our findings indicate that lack of PGC-1α alters mitochondrial redox state and dynamics in RPE and leads to mitochondrial dysfunction, defective autophagy and loss of epithelial integrity.
Commercial Relationships: Mariana Aparecida B. Rosales, None; **Sarah Melissa Jacobo**, None; **Jared Iacovelli**, None; **Magali Saint-Geniez**, None
Support: CNPq PDE process 210474/2014-9 (MR), Grimshaw Foundation (MSG), a Research to Prevent Blindness Unrestricted Grant (MSG) and donations to the Macular Degeneration Research, a program of the Bright Focus Foundation (MSG)

Program Number: 3015 **Poster Board Number:** A0010
Presentation Time: 11:00 AM–12:45 PM
Primate macula and short wavelength cones use glycolysis to fund energy expenditure
Jaimie Hoh Kam, Tobias Weinrich Weinrich, Michael B. Powner, Glen Jeffery. Visual Neuroscience, Institute of Ophthalmology, London, United Kingdom.
Purpose: The retina has the greatest metabolic demand in the body and ageing and disease impact on its central region, which has a distinct architecture and very high cell density. Here we ask how the primate retina and RPE derive energy for cellular function.
Methods: We use eyes from 10 cynomolgous long-tailed monkeys (*Macaca fascicularis*) which were donated at death and were between 4 and 14 years old. Mitochondrial function and glycolysis were analysed using Western blot and immunohistochemistry of different subunits of cytochrome C oxidase and lactate and pyruvate dehydrogenases.
Results: Mitochondrial function was assessed by analysis of COX I, II, III and IV, revealing consistent patterns across the neural retina and RPE. However, the central retina also draws on glycolysis as an energy source shown by the differential presence of lactate dehydrogenase and pyruvate dehydrogenase specifically expressed in the macular. Further, short wavelength sensitive cones (S-cones) may use glycolysis as their main energy source across the retina as they only express lactate dehydrogenase and not pyruvate dehydrogenase. This explains their distinct psychophysical characteristics which saturate prematurely compare to medium and long wavelength sensitive cones. That S-cones use glycolysis is reinforced by their reduced function in diabetes where declining insulin levels will restrict S-cones access to glucose.
Conclusions: Methylglyoxal is a byproduct of glycolysis and this can promote advanced glycation end-products which are toxic. This process may contribute to age related attrition in the central retina and reduced S-cone function in ageing.
Commercial Relationships: Jaimie Hoh Kam, None; **Tobias Weinrich Weinrich**, None; **Michael B. Powner**, None; **Glen Jeffery**, None
Support: BBSRC BB/N000250/1

Program Number: 3016 **Poster Board Number:** A0011
Presentation Time: 11:00 AM–12:45 PM
A non-redundant role for PGC-1β in retinal homeostasis and photoreceptor resistance to oxidative stress induced by light exposure
Casey J. Keuthan, Cassidy M. Chason, John D. Ash. Ophthalmology, University of Florida, Gainesville, FL.
Purpose: The peroxisome proliferator-activated receptor gamma coactivator 1 (PGC-1) family members are considered master regulators of mitochondrial biogenesis and metabolism. Although PGC-1 has been studied in a variety of high-energy tissues, the non-redundancies between the individual PGC-1α and PGC-1β isoforms are not well established. Moreover, the function of PGC-1β in the retina remains largely unknown. This work aimed to further elucidate the role of PGC-1β in photoreceptor protection during aging and in light stress conditions.
Methods: Chx10Cre;PGC-1β$^{f/f}$ mice are conditional knockouts (cKO) of PGC-1β in retinal neurons and Müller glial cells. Littermates not expressing Cre were used as controls. All animals were reared in dim light conditions (<100 lux). For light stress, mice were exposed to light (400 lux or greater). Spectral Domain Optical Coherence Tomography (SD-OCT) was used to study retinal morphology, and retinal function was measured by electroretinography (ERG). Retinas were collected for quantitative RT-PCR (qRT-PCR) to measure mRNA expression. All procedures with animals were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Results: In dim light, young adult (2 months) PGC-1β cKO mice had normal retinas, suggesting these mice had normal retinal development. As cKO mice aged, ONL thinning was observed around 6-9 months by OCT. Around 15 months of age, there was a decrease in cardiolipin synthase and acetyl-CoA carboxylase expression, but no significant changes were observed in oxidative stress markers or mitochondrial-related genes involved in oxidative phosphorylation. Interestingly, young cKO mice were more sensitive to light stress, since they were damaged by light levels that are normally non-damaging in wild-type mice. This included significant outer nuclear layer (ONL) thinning and reduced ERG a-wave amplitudes. Differences in PGC-1α mRNA expression were not obvious in light stressed or aged mice.
Conclusions: Our data suggest a critical role for PGC-1β in photoreceptor survival with age and protection from light stress. This role could not be compensated by PGC-1α. We hypothesize that PGC-1β plays a non-redundant role in mitigating metabolic stress of aging and light-induced oxidative damage. This role may involve maintenance of lipid synthesis and other mitochondrial-related processes.
Commercial Relationships: Casey J. Keuthan, None; **Cassidy M. Chason**, None; **John D. Ash**, None
Support: Funding support to JDA includes NIH R01EY016459-11, Foundation Fighting Blindness, and an unrestricted departmental grant from Research to Prevent Blindness, Inc. Training support to CJK includes NIH T32EY007132.

Program Number: 3017 **Poster Board Number:** A0012
Presentation Time: 11:00 AM–12:45 PM
Genetic loss of the metabolic sensor AMPK results in an accelerated aging of the retina
Lei Xu, Lena T. Phu, Abraham M. Bell, John D. Ash. Ophthalmology, Univ of Florida, Gainesville, FL.
Purpose: 5' adenosine monophosphate-activated protein kinase (AMPK) is an enzyme that plays a central role in regulation of energy and metabolism homeostasis. It is a heterotrimeric complex protein that contains a, b and g three subunits. We have shown previously AMPK catalytic subunit α1 and α2 has non redundant roles in cone photoreceptor function. The goal of this study is to further elucidate the role of AMPK signaling, particularly in aging.
Methods: Neural retina knockout of AMPK α subunit mice (Chx10-Cre; AMPKα1$^{f/f}$ AMPKα2 $^{f/f}$) were examined at various ages. Scotopic and photonic electroretinography (ERG) were used to measure photoreceptor and retinal pigmented epithelium (RPE) function. Retinal structure and morphology was observed by Spectral Domain Optical Coherence Tomography (SD-OCT) and histology. Quantitate qPCR were used to measure the relative ratio of mitochondria DNA (mtDNA) and nuclear DNA copy number. The mRNA expression of genes were measured by qPCR. All procedures with animals were conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.
Results: Neural retina-specific AMPKa knockout mice had normal retinal structure, outer nuclear layer (ONL) thickness. Scotopic, and photopic ERGs were normal at very early age. However, over time cone function was reduced followed by reduced rod function. By 9 months of age, knockout mice had thinner ONLs compared to controls. At 12 months of age, there was extensive changes in retinal morphology, including significant reduction in ONL, inner nuclear layer (INL), as well as inner segment (IS) thicknesses. The RPE was also highly vacuolated. Expression of genes involved in mitochondrial biogenesis and fatty acid metabolism were deceased at 6 months, but there was no change in anti-oxidant defense genes. Relative mitochondrial DNA copy number was reduced in the knockout mice.
Conclusions: In this study, we found retina specific deletion of AMPKa causes extensive changes in the retinal structure with age. This suggests AMPK plays an important role in maintaining retinal function and survival in normal aging. We hypothesize that mitochondria are dysregulated in AMPKa knockout mice, since we found evidence of IS shortening and a reduction in relative mitochondrial DNA copy number.
Commercial Relationships: Lei Xu, None; **Lena T. Phu**, None; **Abraham M. Bell**, None; **John D. Ash**, None
Support: Funding support to JDA include NIH R01EY016459-11, Foundation Fighting Blindness, and an unrestricted departmental grant from Research to Prevent Blindness, Inc.

Program Number: 3018 **Poster Board Number:** A0013
Presentation Time: 11:00 AM–12:45 PM
Fuel exchange between photoreceptors and RPE underlies a retinal metabolic ecosystem
Michelle Giarmarco[1], Mark Kanow[1], Abbi Engel[2], Jianhai Du[4, 3], Jennifer R. Chao[2], James Hurley[1, 2]. [1]Biochemistry, University of Washington, Seattle, WA; [2]Ophthalmology, University of Washington, Seattle, WA; [3]Biochemistry, West Virginia University, Morgantown, WV; [4]Ophthalmology, West Virginia University, Morgantown, WV.
Purpose: The choroid and retinal pigment epithelium (RPE) supply glucose to the outer retina as a vital component of retinal function and health. Photoreceptors (PRs) are highly glycolytic, converting ~90% of glucose they receive to lactate. The RPE is also metabolically active, raising questions about how it can maintain glucose export. We propose that a "metabolic ecosystem" exists in the outer retina, whereby RPE uses PR lactate as fuel, enabling net flow of glucose towards PRs. To test this hypothesis, we examined glucose transporter (GLUT) distribution in mouse retina, assayed

retinal glucose uptake *in vivo*, and measured glucose transport across cultured human fetal RPE (hfRPE) cells using mass spectrometry.
Methods: *Immunohistochemistry:* Adult mouse eyecups were fixed in 4% paraformaldehyde and cut into 20-µm cryosections. Sections were incubated with primary antibodies against glutamine synthetase, arrestin and GLUT1, followed by incubation with fluorescent-tagged secondary antibodies. Sections were imaged via confocal microscopy. In vivo *Glucose Uptake*: A fluorescent glucose analog, 2-NBDG, was administered orally to mice or zebrafish expressing red fluorescent protein in PRs or Müller glia. Following incubation period eyes were collected, and fresh retinal slices were prepared and imaged via confocal microscopy.
hfRPE Glucose Transport: hfRPE cells were grown in transwell filter plates until transepithelial resistance was greater than 200 Ω cm^2. ^{13}C-glucose was added to the basolateral compartment, while increasing concentrations of different metabolites were added to the apical chamber. Transport of ^{13}C-glucose into the apical chamber was quantified using liquid chromatography mass spectroscopy.
Results: GLUT1 is abundant in mouse PRs, and PRs but not Müller glia accumulate 2-NBDG *in vivo*. Together these results indicate that PRs take up outer retinal glucose whereas Müller glia do not. We also found that lactate enhances glucose transport across RPE, suggesting that lactate made by PRs and released into the interphotoreceptor matrix promotes translocation of glucose across the RPE.
Conclusions: Evidence of glucose uptake into PRs, paired with a reliance of RPE on lactate for basal-to-apical glucose transport, supports the existence of a novel metabolic relationship between RPE and PRs. Breakdown of PR or RPE cells' metabolic specificity could contribute to retinal dysfunction in aging or disease.
Commercial Relationships: Michelle Giarmarco, None; **Mark Kanow**, None; **Abbi Engel**, None; **Jianhai Du**, None; **Jennifer R. Chao**, None; **James Hurley**, None
Support: NIH Grant T32EY07031

Program Number: 3019 **Poster Board Number:** A0014
Presentation Time: 11:00 AM–12:45 PM
Analysis of Secretory Lipidomics and Proteomics of Late-Onset Retinal Degeneration iPSC-derived RPE
Kiyoharu J. Miyagishima[1], Ruchi Sharma[1], Katharina Clore-Gronenborn[1], Zoya Qureshy[1], Bokkyoo Jun[2], William C. Gordon[2], Nathan Hotaling[1], Congxiao Zhang[1], Catherine A. Cukras[1], Paul A. Sieving[1], Nicolas G. Bazan[2], Sheldon S. Miller[1], Kapil Bharti[1]. [1]NEI, NIH, Bethesda, MD; [2]Neuroscience Center of Excellence, Louisiana State University Health, New Orleans, LA.
Purpose: Late-Onset Retinal Degeneration (L-ORD) is a rare autosomal dominant disorder caused by a single S163R amino acid substitution in a secreted protein known as CTRP5. The disease phenotype (delayed dark adaptation, sub-RPE deposits) is reminiscent of more prevalent retinal degenerations, thus providing valuable insight into the shared mechanisms underlying their pathogenesis.
Methods: Fibroblasts from two L-ORD affected and two unaffected siblings were reprogrammed into induced pluripotent stem cells (iPSCs) using sendai viral vectors containing the four Yamanaka factors. iPSCs were fully characterized via immunocytochemistry, karyotyping, and spontaneous differentiation of embryoid bodies into all three germ layers. iPSCs were then differentiated into RPE using a developmentally guided protocol. Cell shapemetric analysis was performed using custom software on iPSC-derived RPE stained with ZO-1. Apical media was analyzed for protein and lipid composition.
Results: Although iPSC-derived RPE from patients and unaffected siblings have similar hexagonality (score, side ratio, area ratio), L-ORD patient iPSC-derived RPE tended be more variable in size and on average possessed greater cell areas. Mass Spect analysis revealed elevated sequence coverage of glutathione, cystatin C, keratins, vimentin, actin, and tubulin secreted by patient iPSC-RPE. Lipidomic analysis of patient iPSC-RPE showed higher secretion levels of very long chain- polyunsaturated fatty acids (VLC-PUFAs) corresponding with higher DHA secretion into the apical media compared to unaffected siblings.
Conclusions: The more variable and larger RPE cell sizes in L-ORD patient iPSC-RPE are indicative of cytoskeletal rearrangement perhaps resulting from enhanced uncompensated oxidative stress. In agreement, proteomic analysis of the apical media revealed proteins identified as biomarkers for oxidative stress. Lastly, lipidomic analysis revealed an over-abundance of DHA and VLC-PUFAs in L-ORD patient iPSC-RPE suggesting that elevated levels of DHA may reflect an attempt to set in motion protective instructive signals from DHA and when overproduced may contribute to lipid peroxidation and susceptibility of these cells to oxidative stress-mediated damage.
Commercial Relationships: Kiyoharu J. Miyagishima, None; **Ruchi Sharma**, None; **Katharina Clore-Gronenborn**, None; **Zoya Qureshy**, None; **Bokkyoo Jun**, None; **William C. Gordon**, None; **Nathan Hotaling**, None; **Congxiao Zhang**, None; **Catherine A. Cukras**, None; **Paul A. Sieving**, None; **Nicolas G. Bazan**, None; **Sheldon S. Miller**, None; **Kapil Bharti**, None
Support: Supported by NEI IRP, NEI, EY005121, NIGMS ,GM103340 and the Eye, Ear, Nose and Throat Foundation (NGB)

Program Number: 3020 **Poster Board Number:** A0015
Presentation Time: 11:00 AM–12:45 PM
Role of PPAR-alpha in Retinal Mitochondrial Function
Rui Cheng[1], Elizabeth P. Moran[1, 2], Kelu Zhou[1], Marin Gantner[3], Jian-Xing (Jay) Ma[1]. [1]Physiology, University of Oklahoma Health Sciences Center, Oklahoma City, OK; [2]Department of Ophthalmology, Boston Children's Hospital, Harvard Medical School., Boston, MA; [3]The Lowy Medical Research Institute, La Jolla, CA.
Purpose: The retina is one of the highest energy-consuming tissues in the body. Glucose and lipid are important substrates for retinal ATP production. Peroxisome proliferator-activated receptor alpha (PPARα) is a nuclear receptor regulating fatty acid oxidation (FAO), and PPARα agonists have beneficial effects in retinal diseases of metabolic origin. However, the role of PPARα in retinal energy metabolism remains incompletely understood. This study tested the hypothesis that PPARα is essential for retinal energy metabolism.
Methods: PPARα-deficient (PPARα$^{-/-}$) mice and age and genetic background-matched wild-type (Wt) mice were euthanized. Retinas were freshly dissected. Oxygen consumption rates (OCR) and extracellular acidification rates (ECAR) in response to energy substrates glucose, pyruvate or palmitate were measured using a Seahorse XFe96 Flux Analyzer®. A two-tailed, unpaired Student's t-test was used for statistical analysis.
Results: Palmitate increased OCR in Wt retinas at the age of 4 weeks, but did not change OCR in PPARα$^{-/-}$ retinas, suggesting impaired FAO in the absence of PPARα. To determine if impaired FAO affected glucose metabolism, we measured retinal OCR and ECAR in response to glucose. High glucose decreased the maximal OCR and increased ECAR in 4-week old Wt retinas, suggesting that an abundance of glucose promoted a shift towards glycolytic metabolism. However, although glucose increased ECAR in PPARα$^{-/-}$ retinas, it did not affect maximal OCR, indicating that more glycolytic products may be used for oxidative metabolism in the absence of PPARα. Accordingly, we found that pyruvate did

not significantly affect OCR in Wt retinas, but did increase OCR in PPARα$^{-/-}$ retinas. We further compared mitochondrial function with aging in PPARα$^{-/-}$ retinas by measuring OCR at 4, 8 and 12 weeks of age. We found that beginning at 8 weeks of age, basal and maximal OCR were decreased, and further declined at 12 weeks, suggesting that PPARα deficiency leads to mitochondrial dysfunction.
Conclusions: Endogenous PPARα is essential for retinal energy metabolism. Diabetes-induced PPARα down-regulation may contribute to disturbed energy metabolism in the retina.
Commercial Relationships: Rui Cheng, None;
Elizabeth P. Moran, None; **Kelu Zhou**, None; **Marin Gantner**, None; **Jian-Xing (Jay) Ma**, None
Support: NIH grants (EY018659, EY012231, EY019309, P20GM104934), a JDRF grant (2-SRA-2014-147-Q-R), an IRRF grant and OCAST grants (HR13-076).

Program Number: 3021 **Poster Board Number:** A0016
Presentation Time: 11:00 AM–12:45 PM
Diurnal regulation of peroxisome function in RPE cells, a role for autophagy-dependent turnover
Lauren L. Daniele[1], Jennifer Caughey[3], Anuradha Dhingra[1], Desiree Alexander[2], Nancy J. Philp[4], Kathleen Boesze-Battaglia[1]. [1]Biochemistry, SDM, University of Pennsylvania, Philadelphia, PA; [2]Program in Cell and Molecular Biology, Biomedical Graduate Studies, University of Pennsylvania, Philadelphia, PA; [3]School of Dental Medicine, University of Pennsylvania, Philadelphia, PA; [4]Department of Pathology, Anatomy, and Cell Biology, Thomas Jefferson University, Philadelphia, PA.
Purpose: On a daily basis, photoreceptors shed ~10% of their lipid-rich outer segments (OS), for uptake and degradation by the retinal pigment epithelium (RPE). Ingested OS contain an abundance of very long chain fatty acid (VLCFA) which is oxidized in peroxisomes. β –oxidation of VLCFA produces H_2O_2 that is decomposed by antioxidant enzymes (e.g. catalase). Despite their important role in lipid metabolism, regulation of the biogenesis, activity, and turnover of peroxisomes is not well characterized. The purpose of this study is twofold; we tested the hypothesis that RPE peroxisomes are degraded by selective autophagy by examining peroxisome number and function in RPE of mice lacking ATG5 or LC3B. We also investigated whether peroxisome function and turnover depends on time of day.
Methods: Catalase activity was measured in RPE lysates of *Atg5*$^{\Delta RPE}$, *LC3B*$^{-/-}$ and WT (C57Bl6/J) mice at various times relative to light onset using a fluorescence-based assay and normalized to total catalase as assessed by dot blot. The expression of peroxisome specific membrane proteins Pex14 and PMP70 was assessed by immunohistochemistry (IHC) and Western blot analysis (WB) in RPE from WT, *LC3B*$^{-/-}$ and *Atg5*$^{\Delta RPE}$ mice. Pex14 mediates the import of peroxisomal enzymes, and PMP70 is an ATP binding cassette transporter for long chain fatty acyl CoA intermediates. Dysregulated autophagy was determined by comparing expression of p62, LC3I, and LC3II in *Atg5*$^{\Delta RPE}$ by WB.
Results: In WT RPE, the expression of Pex14 at 3 PM was reduced to ~65% of that at light onset as estimated by WB. In contrast, catalase activity nearly doubled at 3PM relative to light onset. There was no change in Pex14 expression or catalase activity 1 hr before, or after light onset. Pex14 and PMP70-positive puncta appeared more numerous in RPE of *LC3B*$^{-/-}$ and *ATG5*$^{\Delta RPE}$ mouse eyes. Pex14 expression increased by ~20% in *ATG5*$^{\Delta RPE}$ compared to WT in WB analysis. Additionally, p62 immunoreactivity was increased ~6-fold, and the LC3II/LC3I ratio decreased by >50%, in WB of *ATG5*$^{\Delta RPE}$ vs. WT. Like WT, catalase activity was elevated in *LC3B*$^{-/-}$ at 3PM vs. light onset. In contrast to WT, Pex14 levels in *LC3B*$^{-/-}$ did not decrease at 3PM.
Conclusions: Our data suggest that RPE peroxisomes rely on selective autophagy for degradation that depends on time of day, and that diurnal catalase activity may be regulated independently of peroxisome turnover.
Commercial Relationships: Lauren L. Daniele, None;
Jennifer Caughey, None; **Anuradha Dhingra**, None;
Desiree Alexander, None; **Nancy J. Philp**, None;
Kathleen Boesze-Battaglia, None
Support: NIH Grant EY010420

Program Number: 3022 **Poster Board Number:** A0017
Presentation Time: 11:00 AM–12:45 PM
Microtubule-associated protein 1 light chain 3 (LC3) isoforms in RPE: expression and function
Anuradha Dhingra[1], Juan Reyes-Reveles[1], Desiree Alexander[1], Rachel C Sharp[1], Aditi Swarup[2], Hye Jin Kim[3], Janet R. Sparrow[3], Kathleen Boesze-Battaglia[1]. [1]Department of Biochemistry, University of Pennsylvania, Philadelphia, PA; [2]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA; [3]Department of Pathology and Cell Biology, Columbia University, New York, NY.
Purpose: Microtubule-associated protein 1 light chain 3 (LC3) is an essential component of autophagy as well as LC3 associated phagocytosis (LAP), a hybrid degradation pathway utilizing components of canonical autophagy and phagocytosis. Autophagy dependent processes, collectively termed heterophagy, play a key role in RPE homeostasis. LC3 exists as three isoforms in human (LC3A, B, and C), with two of these (LC3A and B) in mouse. Here, we tested the hypothesis that LC3B plays a critical role in maintaining lipid homeostasis in the RPE through degradation of photoreceptor outer segment and recycling of visual pigments.
Methods: *C57Bl6/J* (*WT*) and *LC3BKO* mice (from 2 month to >2 years old) were used. LC3 isoform expression was analyzed by RTPCR and immunoblotting. Biopotigen Spectral Domain Optical Coherence Tomography at 840nm was used to image retina. Bisretinoid levels were measured by HPLC. Fixed frozen retinal sections were stained for neutral lipids with Bodipy 493/503, or immunostained for various antibodies followed by confocal imaging. Lipid turnover as reflected in, β-Hydroxybutyarte (β-HB) released from mouse RPE explants was measured using LiquiColor reagent kit (StanBio).
Results: Quantitative RT-PCR experiments showed that LC3B is the predominant isoform in human RPE, with both LC3A and LC3B expressed at equivalent levels in mouse RPE. In an *LC3BKO* RPE, there appears to be no compensatory upregulation of LC3A transcripts. The levels of bisretinoids A2E and atRALdi-PE were higher in the *LC3BKO* RPE compared to the *WT*. Consistent with this, there appeared to be an increase in neutral lipid staining in the *LC3BKO* RPE. In addition, lipid turn-over, as inferred from mitochondrial ketogenesis was altered: RPE explants from *WT* animals show a peak of β-HB at 2 hours after light onset, whereas in *LC3BKO*, this peak was delayed by 4 hours relative to the *WT*. *LC3BKO* mice show normal retinal layering and thickness, however there was an increase in Iba1 positive microglia in the outer retina in older mice.
Conclusions: LC3B is the predominant LC3 isoform in human RPE, both LC3A and B are expressed in mouse RPE at similar levels and there is no compensatory upregulation of LC3A in the absence of LC3B. Our study provides evidence for the role played by LC3B in lipid homeostasis, as lack of LC3B causes defective

phagocytosis, leading to metabolic imbalance, lipid deposition, and pro-inflammatory microenvironment.
Commercial Relationships: Anuradha Dhingra, None; **Juan Reyes-Reveles**, None; **Desiree Alexander**, None; **Rachel C Sharp**, None; **Aditi Swarup**, None; **Hye Jin Kim**, None; **Janet R. Sparrow**, None; **Kathleen Boesze-Battaglia**, None
Support: NIH EY010420 (KBB), EY026525 (KBB and NJP), EY12951 (JRS)

Program Number: 3023 **Poster Board Number:** A0018
Presentation Time: 11:00 AM–12:45 PM
Loss of GLUT1 in the retinal pigment epithelium does not diminish its function, differentiation or polarity
Ivy S. Samuels[1, 2], Aditi Swarup[3], Craig Beight[1, 2], John Y. Han[3], Jamie Soto[4], E Dale D. Abel[4, 5], Neal S. Peachey[1, 2], Nancy J. Philp[3]. [1]Research Service, Louis Stokes VA Medical Center, Cleveland, OH; [2]Department of Ophthalmic Research, Cole Eye Institute, Cleveland Clinic, Cleveland, OH; [3]Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA; [4]Division of Endocrinology and Metabolism, Carver College of Medicine, University of Iowa, Iowa City, IA; [5]Fraternal Order of Eagles Diabetes Research Center, University of Iowa, Iowa City, IA.
Purpose: The retina is one of the most metabolically active tissues in the body and normal visual function is dependent on a continuous supply of glucose and oxygen. Glucose is transported from the choroid blood supply to the outer retina by GLUT1 transporters expressed in the basolateral and apical membranes of the RPE. In this study, we assessed the impact of conditional deletion of GLUT1 in mouse RPE on the integrity of the outer blood retinal barrier and visual function.
Methods: GLUT1 was conditionally inactivated in the RPE by crossing *Glut1*$^{Flox/Flox}$ mice with the VMD-2-cre mouse to create the *Tg-VMD-2-cre:Glut1*$^{Flox/Flox}$ conditional knockout mouse. The expression of GLUT1 and retinal structure in *GLUT1*$^{Flox/Flox}$, *Tg-VMD-2-cre* and *Tg-VMD-2-cre:Glut1*$^{Flox/Flox}$ mice were compared by optical coherence tomography (OCT), immunohistochemistry and western blot. Retinal and RPE function was assessed by dark-adapted and light-adapted strobe flash electroretinography as well as by direct current-coupled electroretinography.
Results: The expression of GLUT1 in *Tg-VMD-2-cre:Glut1*$^{Flox/Flox}$ mice was found to be patchy with GLUT1 positive cells adjacent to GLUT1 negative cells. The GLUT1 negative RPE did not exhibit changes in expression and polarity of RPE specific genes. No changes in the thickness of the outer nuclear layer were detected and the density of cones did not differ between retinal areas overlying GLUT1 positive or GLUT1 negative RPE cells. No significant differences were found in the dark-adapted strobe flash electroretinogram (ERG), light adapted ERG or direct current-coupled ERG of mice lacking GLUT1 in the RPE.
Conclusions: The reduction of GLUT1 expression in the RPE of the *Tg-VMD-2-cre:Glut1*$^{Flox/Flox}$ mice did not cause changes in visual activity. This suggests that the mosaic pattern of the Cre expression allows sufficient glucose to be transported into the retina to support photoreceptor cell function. The integrity of the barrier properties of the RPE supports the hypothesis that they oxidize lactate and fatty acids, sparing glucose for the outer retina.
Commercial Relationships: Ivy S. Samuels, None; **Aditi Swarup**, None; **Craig Beight**, None; **John Y. Han**, None; **Jamie Soto**, None; **E Dale D. Abel**, None; **Neal S. Peachey**, None; **Nancy J. Philp**, None
Support: This work was supported by VA Merit Awards I01BX002754 (ISS), I01BX002340 (NSP), NIH R01 EY012042 (NJP), P30EY025585, a Foundation Fighting Blindness Center Grant and Research to Prevent Blindness.

Program Number: 3024 **Poster Board Number:** A0019
Presentation Time: 11:00 AM–12:45 PM
Rebalancing RPE energy metabolism slows retinal degeneration
Douglas Vollrath, Melissa Calton. Department of Genetics, Stanford University School of Medicine, Stanford, CA.
Purpose: We previously showed that postnatal loss of RPE oxidative phosphorylation (OXPHOS) in the murine RPE triggers increased aerobic glycolysis, mTOR-mediated dedifferentiation, lipid accumulation and photoreceptor degeneration, supporting a causal role for degradation of RPE OXPHOS in the pathogenesis of age-related macular degeneration (AMD). We next sought to determine whether an imbalance between RPE OXPHOS and aerobic glycolysis, rather than OXPHOS ablation per se, can cause the observed retinal abnormalities. To do so, we assessed the in vivo consequences of enhancing murine RPE aerobic glycolysis in the presence of intact OXPHOS machinery, and subsequently rebalancing metabolism through stimulation of OXPHOS.
Methods: We created mice with postnatal RPE-selective ablation of prolyl-hydroxylase (PHD) genes 1-3 (i.e. Egln1-3) to stabilize hypoxia inducible factor (HIF) and enhance aerobic glycolysis. Retinas of mutant mice and controls were studied at various ages by histology, immunofluorescence, immunoblot, qRT-PCR, funduscopy, electroretinography (ERG), ocular coherence tomography, and ex vivo extracellular flux analysis. Animals were treated by daily intraperitoneal injection of dichloroacetate and oxamate (DO) to stimulate OXPHOS.
Results: The retinas of mutant mice develop normally, but the RPE is histologically abnormal as early as postnatal day 17. The RPE eventually exhibits increased aerobic glycolysis, mTOR activation, hypertrophy, glycogen accumulation, altered pigmentation, and loss of polarity, integrity and characteristic markers, but shows no evidence of increased lipids or induction of VEGF. Levels of HIF-1, but not HIF-2, are increased. ERG amplitudes are depressed and photoreceptors progressively die. DO treatment stimulates OXPHOS in mutant eyecups, and in vivo improves RPE integrity, preserves ERG amplitudes, and slows the loss of photoreceptors, while not altering mTOR activation or the levels of selected RPE markers.
Conclusions: Our results suggest that increased RPE aerobic glycolysis causes RPE and, indirectly, photoreceptor dysfunction in response to stabilization of HIF-1, a protein that could plausibly be activated during AMD pathogenesis. Increasing OXPHOS in a glycolytic milieu mitigates the loss of RPE integrity, and partially preserves photoreceptor function and numbers. Thus, the RPE aerobic glycolysis/OXPHOS balance is critical for retinal homeostasis.
Commercial Relationships: Douglas Vollrath, None; **Melissa Calton**, None
Support: Thome Memorial Foundation and NIH grants R01EY025790 and T32EY20485

Program Number: 3025 **Poster Board Number:** A0020
Presentation Time: 11:00 AM–12:45 PM
Modulation of mitochondrial respiration in hPSC-RPE
Divya Sinha[1, 3], Gurugirijha Rathnasamy[3, 2], Kimberly A. Toops[3, 2], Molly M. Wilson[1], Rasa Valiauga[1], Li Xuan Tan[2, 4], Janis T. Eells[5], Aparna Lakkaraju[2, 4], David M. Gamm[3, 2]. [1]Waisman Center, University of Wisconsin-Madison, Madison, WI; [2]Department of Ophthalmology and Visual Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI; [3]McPherson Eye Research Institute, University of Wisconsin-Madison, Madison, WI; [4]Division of Pharmaceutical Sciences, School of Pharmacy, University of Wisconsin-Madison, Madison, WI; [5]Department of Biomedical Sciences, University of Wisconsin-Milwaukee, Madison, WI.
Purpose: Efficient mitochondrial activity is required to meet the energy demands of RPE, which is a highly metabolically active monolayer of cells possessing multiple critical roles in the outer retina. Given the interest in human pluripotent stem cell-derived RPE (hPSC-RPE) as a source for cell replacement therapies, it is important to investigate mitochondrial health and behavior in hPSC-RPE under relevant environmental conditions, which may in turn improve transplantation strategies.
Methods: Monolayers of RPE were derived from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) using methods established by our lab. hPSC-RPE cells were treated with 10 μM A2E (a retinoid in lipofuscin) for 6 h followed by incubation in fresh culture medium for 48 hours prior to live imaging, and for 72 hours for extracellular flux (XF) assay. Alternatively, cultures were exposed to 50 μM resveratrol for 24 hours directly followed by live imaging and XF analysis. Live-cell imaging and XF assays were used to image mitochondrial structure and measure mitochondrial respiration, respectively. Data analysis was performed using Imaris (Bitplane) for live imaging and Wave (Agilent Technologies) for XF assay.
Results: Mitochondria in hPSC-RPE exist as interconnected networks with readily measurable oxygen consumption rates (OCR) and spare respiratory capacity (SRC). A2E exposure leads to mitochondrial fragmentation, indicative of cellular stress, and increased basal OCR in hPSC-RPE cells (115.97±6.49 pmol/min) compared to untreated hPSC-RPE (94.40±6.98 pmol/min). hPSC-RPE cells treated with resveratrol show highly networked mitochondrial structure compared to control cells. Additionally, resveratrol treatment enhances spare respiratory capacity in hPSC-RPE by up to 1.8-fold compared to untreated cells.
Conclusions: We show that exposure to certain compounds can modulate mitochondrial structure and function in cultured hPSC-RPE. Rigorously monitoring such changes provides a means to study the response of RPE cells to conditions of oxidative stress and to screen for interventions that might enhance mitochondrial activity. In particular, enhanced SRC is critical for meeting increased energy demands under conditions of cellular stress and has the potential to enhance cell survival post-transplant.
Commercial Relationships: Divya Sinha, None; **Gurugirijha Rathnasamy**, None; **Kimberly A. Toops**, None; **Molly M. Wilson**, None; **Rasa Valiauga**, None; **Li Xuan Tan**, None; **Janis T. Eells**, None; **Aparna Lakkaraju**, None; **David M. Gamm**, None
Support: Research to Prevent Blindness Catalyst Award, International Retina Research Foundation, Foundation Fighting Blindness, NIH R01EY024588, Retinal Research Foundation Emmett A. Humble Distinguished Directorship, McPherson Eye Research Institute (Sandra Lemke Trout Chair), Carl and Mildred Reeves Foundation, NIH P30HD03352, Muskingum County Community Foundation, SIG 1S10OD018039-01 for Seahorse XFe96 analyzer, Support Grant P30 CA014520 to SMSSF (core service of the University of Wisconsin Carbone Cancer Center)

Program Number: 3026 **Poster Board Number:** A0021
Presentation Time: 11:00 AM–12:45 PM
Estrogen effects on VEGF-A expression in Human Retinal Pigment Epithelial Cells
Diego Tapias, James Lo, Catherine Chia, Max Kudisch, Elizabeth Winters, Ricardo Lamy, Jay M. Stewart. Ophthalmology, University of California San Francisco, San Francisco, CA.
Purpose: Estrogen has different effects on various tissues depending on the concentration and cell type. It has been shown that 17β-estradiol (E2) can modulate inflammatory response and decrease expression of IL-6 in adult human retinal pigment epithelial (ARPE-19) cells. Tumor Necrotic Factor Alpha (TNF-α) is increased in many retinal diseases involving chronic inflammation and it induces expression of Vascular Endothelial Growth Factor (VEGF)-A by ARPE-19 cells. The purpose of this study is to assess effects of distinct concentrations of E2 on the VEGF-A expression induced by TNF-α on ARPE-19 cells.
Methods: ARPE-19 cells were obtained from the American Type Culture Collection (ATCC) and grown in 12 well culture plates. The cells were divided in 3 groups: cell medium only; cell medium + 1mM E2; cell medium + 2mM E2. After twenty-four hours of incubation, TNF-α (10ng/mL) was added to the medium and the cells were incubated for six more hours. Cells were harvested and the expression level of VEGF-A was assessed in all groups by qPCR using actin as a reference gene.
Results: Compared to the group pre-treated with medium only, we observed a 36% reduction on the expression of VEGF-A in the cell group pre-treated with medium + E2 1mM (p=0.03). The difference observed was not statistically significant (p=0.20) when comparing the group pre-treated with medium + E2 2mM with the group pre-treated with medium only.
Conclusions: Treatment of the ARPE-19 cells with 1mM E2 reduced the impact of TNF-α on the production of VEGF-A. The reduction observed at a higher E2 concentration was not significant. Systemic and local levels of estrogens may play an important role in preventing the progression of retinal diseases involving chronic inflammation and VEGF-A expression. The differential E2 response needs further investigation.
Commercial Relationships: Diego Tapias; **James Lo**, None; **Catherine Chia**, None; **Max Kudisch**, None; **Elizabeth Winters**, None; **Ricardo Lamy**, None; **Jay M. Stewart**, None
Support: NIH-NEI EY002162 - Core Grant for Vision Research; Research to Prevent Blindness Unrestricted Grant.; That Man May See

Program Number: 3027 **Poster Board Number:** A0022
Presentation Time: 11:00 AM–12:45 PM
Abnormal blood vessel formation due to VEGFR2-expressing exosomes released from stressed RPE cells, is influenced by autophagy
Francisco J. Romero[1], Sandra Atienzar[1], Gemma Serrano-Heras[2], Carmen Ruiz de Almodovar[3], Jorge M. Barcia[1], Javier Sancho-Pelluz[1]. [1]School of Medicine, Univ Catolica de Valencia, Valencia, Spain; [2]General University Hospital of Albacete, Albacete, Spain; [3]University Heidelberg, Heidelberg, Germany.
Purpose: Autophagy and exosome secretion are known to play important roles in the cell, in a variety of physiological and disease states, including the development of neovascular age-related macular degeneration (AMD). Indeed, previous studies pointed out that these cellular mechanisms share common pathways of activation.
Methods: Low oxidative damage in ARPE-19, a human retinal pigment epithelium (RPE) cell line, enhances both autophagy and exosome biogenesis. Moreover, oxidative stress modifies the protein and genetic exosomal cargo of exosomes, possibly affecting the fate of surrounding cells. In order to understand the connection between these two mechanisms, stressed ARPE-19 cells were treated with a siRNA for Atg7, protein key for the formation of autophagosomes.
Results: Subsequently, we observed the formation of multivesicular bodies and the release of exosomes, which happened to be decreased in number. RPE-released exosomes contained VEGFR2 as part of their cargo. This receptor of the vascular endothelial growth factor (VEGF) – which is critical for the development of new blood vessels during angiogenesis in wet AMD – was more present in exosome populations released from ARPE-19 cells facing oxidative stress. While stressed-RPE exosomes enhanced tube formation of endothelial cells, when VEGFR2 was silenced in ARPE-19 cells, resulting exosomes became ineffective, not being able to influence angiogenesis. Moreover, vessel sprouting under stressed-RPE exosome influence seems to follow a non-dependent VEGF pathway.
Conclusions: Previous studies pointed out that RPE cells under stressful conditions enhance autophagy and exosome secretion. We believe that these two mechanisms are involved in the progress of abnormal tube formation. Apparently both mechanisms work together to release different proangiogenic factors that will result in the excessive growing of anomalous vessels, which are extremely fragile and leaky.
Commercial Relationships: Francisco J. Romero, None; **Sandra Atienzar**, None; **Gemma Serrano-Heras**, None; **Carmen Ruiz de Almodovar**, None; **Jorge M. Barcia**, None; **Javier Sancho-Pelluz**, None
Support: Grant Prometeo 94/2016, Generalitat Valenciana, Spain.

Program Number: 3028 **Poster Board Number:** A0023
Presentation Time: 11:00 AM–12:45 PM
Contact of Rod Outer Segment Tips with the RPE Triggers Glucose Transport From the RPE to Cone Photoreceptors for OS Synthesis and Function
Wei Wang[1], Henry J. Kaplan[1], Douglas C. Dean[1, 2]. [1]Department of Ophthalmology, University of Louisville, Louisville, KY; [2]James Graham Brown Cancer Center, University of Louisville, Louisville, KY.
Purpose: Retinitis Pigmentosa (RP) is the most frequent cause of hereditary visual loss in adults. Mutations most frequently target rod photoreceptors and rod loss results in nyctalopia and a constricted visual field. But, the major visual disability in RP results from subsequent cone photoreceptor dysfunction, leading to diminished high resolution central vision that is required for tasks such as reading and facial recognition. Previously, we demonstrated that transplanted rod precursors could restore host cone function. We linked rod mutation to a failure to transport glucose from the RPE to cone photoreceptors, and provided evidence that directly injecting glucose into the subretinal space could bypass this defect and restore cone OS synthesis and function. Unraveling the mechanism through which rods are required for transport of glucose to cones might reveal a pathway to preserve/restore central cone vision in RP patients with rod mutations.
Methods: Using both pig and mouse models of RP carrying a P23H *Rho* mutation model of RP, we injected purified OS tips from WT animals or glucose into the subretinal space of animals where cone OS synthesis and function was diminished. Then we analyzed fluorescent glucose transport, full field ERG, multifocal ERG, and cone OS length.
Results: We provide evidence RPE contact with long rod OS tips triggers release of glucose from the RPE into the subretinal space, where it is taken up by cones for use in new OS synthesis, and thus, function. As OS formation diminishes in mutant rods in RP, glucose is no longer transported from the RPE to cones, leading to diminished cone OS synthesis and function.
Conclusions: Injection of WT OS tips to trigger glucose release from the RPE or direct injection of glucose into the subretinal space can bypass the glucose transport defect to cones, and thereby restore cone OS synthesis and function in RP models.
Commercial Relationships: Wei Wang, None; **Henry J. Kaplan**, None; **Douglas C. Dean**, None
Support: Research to Prevent Blindness, American Health Assistance Foundation, NIH Grants (P20 RR018733 and EY015636) and The Commonwealth of Kentucky Research Challenge.