Submit your application to Dr. Denise Inman

1. **Title:** Dendritic arbor and synapse alterations in glaucoma-related neurodegeneration  
   **PI:** Denise Inman, PhD; Assistant Professor in the Department of Pharmaceutical Sciences  
   **Location:** Research and Graduate Education Building 100-4a

2. **Abstract**  
   Retinal ganglion cells (RGCs) are the projection neurons that convey the visual signal from the eye to the brain. These cells are susceptible to pressure-invoked damage in patients with primary open angle glaucoma. RGC axons and dendrites undergo significant change during the course of glaucoma development, though it is not clear whether dendritic changes precede axonal ones. Understanding whether dendrite or axon changes drive disease process is important to develop appropriate glaucoma therapeutic strategies. We have recently used a ketogenic diet to protect RGC structure and function in mice with glaucoma. Among the several possible mechanisms of RGC protection with the diet, we have determined that limiting inflammation and upregulating energy substrate transporters are necessary for RGC survival. We hypothesize that maintaining dendrites and their synapses also contributes to RGC survival and function, and we propose to test whether the ketogenic diet contributes to dendrite and synapse integrity.

3. **Significance**  
   Earlier work in our lab demonstrated that mice developing glaucoma show decreased ATP levels in the optic nerve (1). The magnitude of the ATP decrease was inversely correlated with age and intraocular pressure (IOP), two indices that are risk factors for glaucoma development. The higher the age and IOP, the lower the ATP levels. These ATP decreases were also accompanied by decreases in the transporters that move energy substrates such as glucose and lactate from the bloodstream into the axons and glia of the optic nerve. In order to determine how critical the feeding of mitochondria is to ATP generation, we put the glaucoma mice on a ketogenic diet, thereby increasing the amount of lactate and other monocarboxylates (such as ketone bodies) in the bloodstream.

   The ketogenic diet was able to prompt upregulation of monocarboxylate transporters, allowing the mitochondria access to lactate and ketone bodies, the result of which was protection of RGC structure and function. There were significantly more RGCs in the retinas from mice on the ketogenic diet, and those RGCs showed improved activity and axon transport than RGCs in mice on regular chow. The mechanism of RGC protection includes signaling through HCAR1, a monocarboxylate receptor on microglia, that limits inflammation in the retina and optic nerve. The ketogenic diet also worked through the mTOR complex 1 (mTORC1) to upregulate mitochondrial biogenesis, another way in which the diet supported RGC function. A recent study demonstrated that mTORC1 is necessary to promote dendrite growth in RGCs after optic nerve crush (2). The engagement of mTORC1 with the ketogenic diet, and its role in the regrowth of damaged dendrites with optic nerve damage, led us to hypothesize that the ketogenic diet would also promote dendrite growth or maintenance in RGCs in glaucomatous retina. If we test this hypothesis and learn that dendrites and synapses are maintained to normal levels in the mice on the ketogenic diet, then we will also determine if mTORC1 is necessary for the effect. These findings will ultimately allow us to further refine the mechanism of ketogenic diet protection of RGCs, but also to identify potential therapeutic targets for glaucoma treatment.
4. Goals and objectives
The goal of these studies is to determine the mechanism by which dendrites and synapses are maintained during glaucoma pathology.

The objective of these proposed studies is to evaluate RGC dendrites and synapses from retinas of mice with glaucoma who are fed either a ketogenic diet or normal chow. Once we observe any changes in dendritic arbors or synapse number in mice on the ketogenic diet, then we will determine if those changes occurred as a result of mTORC1 activity.

5. Investigative methods to be used

Glaucoma Modeling We have two models of glaucoma regularly used in the laboratory, 1) an inbred strain (DBA/2) that spontaneously develops glaucoma, and 2) a model of ocular hypertension induced by the injection of magnetic beads into the anterior chamber of the eye. We will use both of these models to test our hypothesis. Half of these mice will be fed a ketogenic diet and the other half control chow.

Tissue Processing Mice with glaucoma and age-matched controls will be perfused, and the eyes, optic nerves, and brains collected. These tissues will be sectioned on a cryostat or microtome in preparation for immunohistochemistry.

Immunofluorescence Analysis Sectioned retina will be immunolabeled using antibodies against dendrites (MAP2), post-synaptic density proteins (e.g., PSD-95) and pre-synaptic proteins (e.g., VGLUT1) in order to evaluate dendritic arbors and synapses in ketogenic diet or control chow mice. The labeled sections will be analyzed using colocalization scripts in ImageJ software. We will also analyze for total pixel number for each label, comparing across groups.

RNA Silencing We will use siRNA to interfere with mTORC1 activation by knocking down the mRNA for Raptor, an essential protein in the mTORC1 complex. siRNA for Raptor will be injected into the posterior chamber of the eye. If Raptor knockdown results in dendrite arbor loss and synapse loss compared to control, then we will have demonstrated that maintenance of dendrites and synapses occurs through mTORC1 activation.

6. Proposed method of data analysis
For the proposed studies, differences between individual groups will be analyzed for statistical significance using the appropriate test. Data following a normal distribution will be analyzed using 2 x 2 factorial analysis of variance (ANOVA) with experimental group (experimental v. control) and age (3 and 10-month) serving as between subjects’ factors and outcome measures serving as the dependent variables. Post-hoc comparisons using Tukey’s HSD tests will be used to define relationships revealed by significant main effects and/or strain by age interactions. Any difference with a probability value less than 0.05 will be considered statistically significant.

7. Significance of anticipated findings
The results of these studies will allow us to determine whether the ketogenic diet has maintained dendritic arbors and synapses within the inner plexiform layer, the synaptic layer within the retina of the RGCs. The siRNA experiment will determine whether the dendrite and synapse maintenance depends upon mTORC1 activation. If our hypothesis is correct, then we will have identified an important therapeutic target for the treatment of glaucoma.
Student Training/Mentoring Plan

• The summer fellow will be largely trained by the PI, with some oversight and/or training by graduate students in the lab. The summer fellow will have weekly one-on-one meetings with the PI, but will be encouraged to come to the PI at any time with questions or to request help with any research or scholarly matter. The PI oversees weekly lab meetings attended by lab members, at which one or more individuals presents data or poses a problem for the group to solve collectively. It is through these meetings that the student will develop a sense for how to think about and approach the research enterprise. The PI will assign reading to the summer fellow that will form the foundation of understanding in the field of research study. Using this basis, the PI will help the fellow identify open questions or unmet needs in the field. It is hoped that the fellow will find one or more of these unmet needs interesting enough for them to want to pursue an answer. These questions will grow into hypotheses based on further discussion. The PI will encourage the fellow to consider each hypothesis, its feasibility given the tools and time at hand, and then decide which one fits all of the constraints. The fellow and PI will then discuss the possible ways to test the hypothesis by drawing on the literature and past experience. Given the literature reviewed and the research undertaken while the hypothesis development plays out, the fellow will likely have a research question that dovetails nicely with the proposed research as outlined here. Finally, the fellow will be encouraged to attend any seminars that take place on campus during the fellowship period, as well as the neurodegeneration data club the PI currently attends. The summer fellow will also spend time writing up his research results under the guidance of the PI, so that the fellow is well prepared for the summer fellow poster session to occur in the Fall.

• Resources available to the student include a desktop lab computer, a desk in the write-up space belonging to the PI, and access to the core facilities and library at NEOMED.

• The research will be conducted in the Research and Graduate Education building, in the Department of Pharmaceutical Sciences. The PI has lab space, a surgical suite and access to microscopes that reside there.

References