HINDLIMB SUSPENSION AS A MODEL TO STUDY OPHTHALMIC COMPLICATIONS IN MICROGRAVITY STATUS REPORT: OPTIMIZATION OF RAT RETINA FLAT MOUNTS STAINING TO STUDY VASCULAR REMODELING

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Preliminary data from a prior tissue-sharing experiment has suggested that early growth response protein-1 (Egr1), a transcription factor involved in various stress responses in the vasculature, is induced in the rat retina after 14 days of hindlimb suspension (HS) and may be evidence that mechanical stress is occurring secondary to the cephalad fluid shift. This mechanical stress could cause changes in oxygenation of the retina, and the subsequent ischemia- or inflammation-driven hypoxia may lead to microvascular remodeling. This microvascular remodeling process can be studied using image analysis of retinal vessels and can then be quantified by the VESsel GENeration Analysis (VESGEN) software, a computational tool that quantifies remodeling patterns of branching vascular trees and capillary or vasculogenic networks. Our project investigates whether rodent HS is a valid model to study the effects of simulated-weightlessness on ocular structures and their relationship with intracranial pressure (ICP). One of the hypotheses to be tested is that HS-induced cephalad fluid shift is accompanied by vascular engorgement that produces changes in retinal oxygenation, leading to oxidative stress, hypoxia, microvascular remodeling, and cellular degeneration. We have optimized the procedure to obtain flat mounts of rat retina, staining of the endothelial lining in vasculature and acquisition of high quality images suitable for VESGEN analysis. Briefly, eyes were fixed in 4% paraformaldehyde for 24 hours and retinas were detached and then mounted flat on microscope slides. The microvascular staining was done with endothelial cell-specific isolectin binding, coupled to Alexa-488 fluorophore. Image acquisition at low magnification and high resolution was performed using a new Leica SP8 confocal microscope in a tile pattern across the X,Y plane and multiple sections along the Z-axis. This new confocal microscope has the added capability of dye separation using the Linear Unmixing method and allows us to remove the autofluorescence originating from the photoreceptor layer. In summary, we have an improved method for studying the retinal microvasculature that will provide an increase in the quality of images captured and will be applied throughout the various animal cohorts of the recently-initiated study that will evaluate rodent HS as a model to study ophthalmic complications in microgravity.