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 
occuring 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 be 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 opthalmic complications in 
microgravity.