

Cell suspensions were stained with fluorochrome-conjugated monoclonal antibodies for flow cytometry. Statistical significance was determined using a two-tailed Student t test or one way ANOVA multiple comparisons test. The minimal level of confidence deemed statistically significant was  $p < 0.05$ . RESULTS/ANTICIPATED RESULTS: Preeclampsia resulted in lower body and heart masses in offspring. Although T cell populations in the thymus were not altered in preeclampsia offspring, total T cells, Thelper, and cytotoxic T cells were elevated. Total B and isotype-switched B cells were increased in offspring of preeclampsia. Total dendritic cell percentages were not changed in offspring of preeclampsia, however, total anti-inflammatory markers on dendritic cells were reduced. Lastly, offspring of preeclampsia had a reduction in microglia and astrocytes within the brain. DISCUSSION/SIGNIFICANCE: Our study could establish including in utero data in predicting future disease risk, addressing gaps in understanding rising rates of cardiovascular and behavioral diseases. It also uncovers the impact of preeclampsia on early immune programming and reduced glial cell populations, potentially affecting cognitive and behavioral development.

### Validation of a Novel CSF-Based Biomarker of Mitochondrial Function<sup>†</sup>

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OBJECTIVES/GOALS: Determine the exosome mitochondrial DNA (mtDNA) copy number in cerebrospinal fluid (CSF) as a measure of neuronal mitochondrial integrity in patients with subarachnoid hemorrhage (SAH). Determine the patterns of beta amyloid and tau protein biology in CSF of SAH patients and correlate those measures with the clinical status of the SAH patients. METHODS/STUDY POPULATION: The CSF is collected from SAH patients undergoing ventriculostomy-based continuous CSF drainage. Adults from all ethnicities and sex are included in this study. The exosomes are isolated from CSF samples using a precipitation method and particle count and size are measured using NanoSight. The DNA is extracted using an exosomal DNA isolation kit (XCF kit). The CSF mtDNA copy number is measured using digital drop PCR with mitochondrial DNA primers. The levels of beta-amyloid (a-beta-40 and -42) and tau protein in CSF are measured using a sensitive ELISA-based assay. A quantitative evaluation of mitochondrial DNA copy number, clinical status of the SAH patients and beta amyloid, and tau protein levels will be conducted and reported. RESULTS/ANTICIPATED RESULTS: Preliminary results of four CSF samples showed similar patterns in CSF exosome particle number, particle size and exosomal mtDNA copy number in relation to samples from the admission day. Particle number decreased with time while particle size increased. More patient samples will be analyzed to confirm the patterns. We anticipate that mtDNA copy number will correlate with brain beta-amyloid and tau protein levels. Moreover, we anticipate that the clinical status of the SAH patients will associate with the mtDNA copy number. We specifically predict that higher mtDNA copy number levels will correlate with better clinical outcomes. DISCUSSION/SIGNIFICANCE: Mitochondrial function is critical to brain health, but we lack effective ways to monitor this parameter. Here we focus on a CSF based biomarker,

exosome-derived mtDNA, which is intended to reflect the integrity of brain mitochondria. As bioenergetic metabolism influences beta amyloid and tau biology, predicting those levels are important.

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### Identification of novel plasma protein biomarkers for diagnosis and prediction of Alzheimer's disease in African Americans\*<sup>†</sup>

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OBJECTIVES/GOALS: To identify novel panel of plasma protein biomarkers to improve prediction and diagnosis of Alzheimer's disease (AD) for African Americans (AA), who are at greater risk of developing AD compared to non-Hispanic White individuals but are underrepresented in AD research. METHODS/STUDY POPULATION: Pre-existing plasma samples from 460 AA individuals with clinical diagnoses of AD, cognitively unimpaired (CU), mild cognitive impairment (MCI), or dementia with Lewy bodies (DLB) will undergo untargeted proteomics using the SomaScan assay, where modified single stranded DNA aptamers bind to protein targets which are quantified by DNA microarray. Protein expression levels will be compared between diagnostic groups to identify differentially expressed proteins. Additional clinical, genetic, and lifestyle factors will be compared with protein expression when available. Proteins of interest, identified by differential protein expression analysis results, will be included in receiver operating characteristic analyses to identify the optimal set of proteins for diagnostic classification. RESULTS/ANTICIPATED RESULTS: A pilot experiment utilizing plasma from 40 individuals identified multiple differentially expressed proteins (DEPs) between AD and non-AD groups. Eight proteins were nominated from the differential protein analysis into a receiver operating characteristic (ROC) analysis based on pvalue and previous implication in AD genome wide association studies. Proteins involved in microglial activation, neuronal adhesion, cell proliferation, and innate immunity were nominated. The ROC model achieved 100% classification accuracy of AD and CU groups using age, sex, and the eight nominated proteins. It is expected that there will be more significant associations when utilizing the full cohort of 460 AA and that DEPs between AD, CU, MCI, and DLB will be identified. DISCUSSION/SIGNIFICANCE: The nomination of a novel panel of plasma biomarkers developed from an AA cohort will directly serve the AA community by improving access to an early and accurate diagnosis of AD. Access to improved prediction and diagnosis will likely improve disease management, thus improving patient outcomes and decreasing burden on families and caregivers.

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### Ischemic conditioning improves dynamic balance during treadmill walking in chronic stroke survivors

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OBJECTIVES/GOALS: Evaluate the use of IC to improve stroke survivors' capacity for reactive stepping and adapt their gait cycles in

response to a difficult walking environment. We hypothesize that IC will improve stroke survivors' protective stepping response via improvements in muscle activation and motor learning. **METHODS/STUDY POPULATION:** Stroke survivors have an impaired capacity for protective stepping. Decreased paretic muscle activation results in increased reaction time and reduced force generation. Ischemic conditioning (IC) is a vascular stimulus which improves motor performance in chronic stroke. It is performed by delivering transient, intermittent bouts of ischemia to a limb. It has been demonstrated that IC increases muscle activation post-stroke. 9 chronic stroke survivors completed 3 testing sessions and 7 intervention sessions. Participants walked on an instrumented treadmill and were perturbed unilaterally every step at the waist via a cable pulley system. Kinetic and kinematic data were collected. Step width was measured as the difference in position of the heel markers at the instant of heel strike in the frontal plane. **RESULTS/ANTICIPATED RESULTS:** After one and seven sessions of IC, controls did not alter their responses from baseline testing, but stroke survivors increased their step width by an average of 15% and 23% respectively. **DISCUSSION/SIGNIFICANCE:** Ischemic conditioning may be a useful intervention to improve stroke survivors' ability to adapt their paretic foot placement in response to lateral perturbations during gait. Interventions which optimize muscle activation and neural adaptation could significantly improve balance post-stroke.

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### Pharmacologic Modulation of Endothelial Cell Autophagy During Hypoxic Cold Storage and Reperfusion: Harnessing the Power of 'Self-Eating,' as a Pre-Treatment Strategy for Donor Organs

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**OBJECTIVES/GOALS:** Donor hearts are transported in cold storage (CS) and undergo ischemia-reperfusion injury (IRI) when transplanted. IRI injures microvascular endothelial cells (EC), heightens the immune response, and has been associated with increased autophagy. We aim to understand the changes in autophagy during CS and IRI and its impact on EC immunogenicity. **METHODS/STUDY POPULATION:** To study autophagy changes during IRI, immunoblotting for autophagy markers was performed in mouse cardiac ECs (MCECs) lysates. MCECs were in a cold preservation solution in a hypoxic chamber for 6 hours(h) and warm conditions with culture medium for 24 h. MCECs, under standard conditions, served as controls. Secreted interferon-gamma (IFN- $\gamma$ ) levels were quantified via ELISA to study autophagy and EC immunogenicity. MCEC-sensitized CD8+ T-cells were isolated from C57BL/6 spleens and co-cultured with MCECs pre-treated for 16 h with rapamycin or starvation, autophagy inducers, or chloroquine, an autophagy inhibitor under normal or IRI conditions. MCECs without any treatment served as controls. **RESULTS/ANTICIPATED RESULTS:** To determine autophagy levels in IRI, immunoblotting of MCEC lysates

revealed a significant increase ( $P < 0.01$ ) in the established autophagy marker, LC3, at early time points post-reperfusion compared to NT conditions, indicating more autophagosome formation during CS and IRI. To assess the role of autophagy in EC immunogenicity, the co-culture experiment revealed that autophagy induction in MCECs under NT and HCS conditions with rapamycin had a 74.9-fold and 51.5-fold reduction of IFN- $\gamma$  (pg/mL), respectively, compared to the non-treated controls. In contrast, autophagy inhibition in MCECs with chloroquine resulted in 1.82-fold increase of IFN- $\gamma$  compared to untreated controls. This suggests a protective role of autophagy in ECs during IRI. **DISCUSSION/SIGNIFICANCE:** We observed that autophagy may be protective during IRI by mitigating EC immunogenicity. Thus, pharmacologically modulating microvascular EC autophagy in donor hearts prior to transplantation may mitigate insults incurred during CS and IRI.

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### Cellular senescence contributes to inflammation and disease progression in an animal model of multiple sclerosis

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**OBJECTIVES/GOALS:** Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system. MS affects more than two million people worldwide, resulting in physical disability, cognitive impairment, and decreased quality of life. We are investigating the role of senescent cells, a hallmark of aging, in inflammation and disease progression in MS. **METHODS/STUDY POPULATION:** Female mice on a C57BL/6 background were subjected to the Hooke Laboratory EK-2110 experimental autoimmune encephalomyelitis protocol (EAE; n=10) to induce hind limb paralysis, and matching control mice received no injections (naïve; n=10). Immunofluorescent staining was used to visualize senescence cells and their localization in spinal cord tissue sections from naïve and EAE mice based on antibody detection of cell surface or intracellular proteins. Tissue sections from each group were analyzed in duplicates for each antibody (n=3-4/group). Flow cytometry was performed to immunophenotype the senescence cells using conjugated fluorescent antibodies to cell surface or intracellular proteins (n=5/group). **RESULTS/ANTICIPATED RESULTS:** Immunostaining demonstrated an increase in the cell senescence markers p16 (15-fold; unpaired t-test,  $p=0.01$ ; n=3) and p21 (15-fold; unpaired t-test,  $p=0.003$ ; n=3) in the spinal cord of EAE mice compared to naïve mice. Next, we showed that p16+ and p21+ cells increase with disease progression in the meninges adjacent to the ventral demyelinating lesions (one-way ANOVA,  $p=0.0009$ ; n=3/group). We further found that 30±9.7% of M2 macrophages, a subset of myeloid cells, express p21 in the spinal cord of EAE mice by using flow cytometry analysis compared to only 5±1.6% in naïve mice (unpaired t-test,  $p=0.002$ ; n=5/group). **DISCUSSION/SIGNIFICANCE:** Our findings demonstrate that senescent cells accumulate in the meningeal compartment following EAE induction, suggesting that decreasing senescent cell burden is a promising avenue in delaying disease progression and preventing neuroinflammation in MS.