

(nitrate-depleted beetroot juice, 0.05 mmol, 140 mL). Following supplementation, participants reported to the lab for measures of vascular function in the lower limb. Doppler ultrasonography was used to measure flow-mediated dilation (FMD) and post-occlusive reactive hyperemia (RH) of the superficial femoral artery in response to a 5-min bout of leg ischemia. RESULTS/ANTICIPATED RESULTS: FMD did not differ between the nitrate-rich ( $2.87 \pm 2.01\%$ ) and placebo ( $2.24 \pm 1.69\%$ ) conditions ( $p = 0.48$ ;  $d = 0.35$ ). Furthermore, peak RH did not differ between the nitrate-rich ( $1503 \pm 443$  ml/min) and placebo ( $1762 \pm 414$  ml/min) conditions ( $p = 0.36$ ;  $d = 0.46$ ). DISCUSSION/SIGNIFICANCE: These preliminary results suggest that dietary nitrate supplementation in the form of beetroot juice does not improve vascular function in individuals with prediabetes.

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### **In Vitro Uptake of Harmful Algal Bloom Toxin Microcystin-LR in Human Placental Cells**

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OBJECTIVES/GOALS: Harmful algal blooms (HABs) are increasing in both frequency and intensity due to climate change. HABs release the toxin microcystin-LR (MC-LR) which enters cells via organic anion-transporting polypeptides (OATPs). In this study, we sought to assess the ability of MC-LR to accumulate in trophoblasts, potentially disrupting placental functions. METHODS/STUDY POPULATION: Intracellular accumulation of MC-LR at exposure concentrations of 0.1, 1, and 10  $\mu$ M over 6 hrs was evaluated in immortalized JAR placental cytotrophoblasts. Western blotting was used to evaluate protein-bound MC-LR accumulation in JAR cells. The function of OATP transporters in JAR cells was determined by pre-incubating cells with 10 $\mu$ M cyclosporin A, a general OATP inhibitor for 1 hr, and then incubated with 1 $\mu$ M OATP substrate fluorescein for up to 40 min. Fluorescence of fluorescein was measured at Ex/Em: 494nm/515nm by spectrophotometry. RESULTS/ANTICIPATED RESULTS: A concentration-dependent increase of MC-LR bound proteins in JAR cells was observed at 6 hrs with the greatest intracellular accumulation of MC-LR at 10 $\mu$ M. In the transporter experiments, a significant decrease of fluorescein uptake by up to 45% into JAR cells was observed following cyclosporin A inhibition of OATPs. These findings are consistent with the functional expression of OATP transporters in JAR placenta cells. Ongoing studies are evaluating whether the cyclosporin A-mediated inhibition of OATPs also inhibits the uptake of MC-LR. DISCUSSION/SIGNIFICANCE: Although MC-LR is well-known for its hepatotoxic and neurotoxic effects, there is growing interest in examining its potential adverse impacts on female reproductive health, particularly during pregnancy. Active uptake of MC-LR into the placenta could interfere with placental and fetal development.

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### **Examining Temporal Links Between Distinct Negative Emotions and Tobacco Lapse During A Cessation Attempt**

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OBJECTIVES/GOALS: Negative emotions (NE) play a pivotal role in addiction-related processes, including tobacco lapse during a quit attempt. Some NEs (e.g., shame, guilt) are posited to lead to a

spiraling effect, whereby lapse predicts increased NEs leading to further lapse. This study goal is to examine associations between NEs and lapse. METHODS/STUDY POPULATION: This study examined associations between tobacco lapse and 13 distinct NEs among people who use tobacco and are trying to quit in two tobacco cessation studies. In Study 1, 220 adult (ages 18-74) cigarette users who identified as Black (50% female) participated in a 14-day study where ecological momentary assessment (with assessments approximately every 4 hours) was used to assess emotions and lapse in real-time and real-world settings. In Study 2, 288 adult (ages 18-71) cigarette users who were low socioeconomic status (51% White, 14% Black, 10% Hispanic, 49% female) participated in a 14-day study with the same study protocol as Study 1. Between and lagged within-person associations testing links between distinct NEs and lapse were examined with multilevel modeling with logistic links for binary outcomes. RESULTS/ANTICIPATED RESULTS: Results from Study 1 suggested that at the between-person level, disgust (OR =1.22, CI: 1.05, 1.42), nervousness (OR=1.23, CI:1.05,1.43), guilt (OR=1.40, CI: 1.16,1.69), and sadness (OR=1.18, CI:1.02,1.36) were predictive of higher odds of lapse, and at the within-person level, shame (OR=1.23, CI:1.04,1.45) was associated with higher odds of lapse. Results from Study 2 were similar and suggested that at the between-person level, disgust (OR=1.35, CI: 1.16, 1.56) and guilt (OR=1.88, CI:1.07,3.30), and at the within-person level, shame (OR =1.31, CI:1.10,1.55), were associated with higher odds of lapse. DISCUSSION/SIGNIFICANCE: The present study uses real-time, real-world data to demonstrate the role of distinct NEs on momentary tobacco lapse and helps elucidate specific NE that hinder the ability to abstain from tobacco use during a quit attempt. Results suggest that disgust, guilt, and shame play consistent roles in predicting lapse among diverse samples of tobacco users.

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### **A CRISPR/dCas9 Epigenetic Therapeutic Approach for CASK-Related MICPCH\***

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OBJECTIVES/GOALS: CASK-related microcephaly with pontine and cerebellar hypoplasia (MICPCH) is a rare X-linked neurodevelopmental disorder caused by mutations in calcium/calmodulin-dependent serine protein kinase (CASK). We aim to rescue CASK expression via an CRISPR/dCas9 epigenetic therapeutic and create iPSC-based CASK relevant in vitro model systems. METHODS/STUDY POPULATION: As females have two X-chromosomes, disease causing mutations present with a 50/50 expression of mutant and wildtype, due to the mosaicism caused by random X-chromosome inactivation (XCI). This project will adapt an established CRISPR/dCas9 epigenetic approach to rescue expression from the silenced, wild-type CASK allele. We aim to accomplish this through testing different dCas9 orthologues and a guide RNA screen targeting the CASK promoter. Constructs will be tested for optimal targeting efficacy in vitro and assessed via RT-qPCR. Additionally, epigenetic modifications from our approach will be analyzed through bisulfite sequencing. We also aim to apply this epigenetic rescue technology in disease relevant cell lines and eventually in engineered patient mutation iPSC-derived neurons. RESULTS/ANTICIPATED RESULTS: Our results show the ability to target CASK and assess gene expression changes with CRISPR/dCas9 paired with an epigenetic modifier and transcriptional activator. Additionally, our fibroblast model with nonpathogenic single

nucleotide polymorphisms within CASK allow for allele specific analysis of our targeted reactivation. We anticipate that following an increase of CASK expression, there would be a decrease in region specific promoter methylation. Further, with the identification of clinically described disease-causing point mutations that result in a loss of function of CASK protein, induction of the mutant sequence onto a healthy cell background will result in a similar reduction of CASK protein in our cell model. **DISCUSSION/SIGNIFICANCE:** This project will demonstrate the first therapeutic avenue for CASK-related MICPCH, and the potential to utilize targeted X-reactivation as a platform approach for X-linked disorders. Further, investigation of smaller dCas9 orthologues prepares our approach for future translational applications such as packaging into AAV for delivery.

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### **Clinical and Translational Research at The University of Florida College of Veterinary Medicine**

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**OBJECTIVES/GOALS:** To demonstrate a successful example of clinical and translational research at a busy veterinary teaching hospital and highlight a collaborative effort in Comparative Oncology between the University of Florida's (UF) Colleges of Medicine and Veterinary Medicine. **METHODS/STUDY POPULATION:** The UF College of Veterinary Medicine (CVM) is a full-time teaching hospital with multiple departments actively recruiting patients for clinical trials. These departments include but are not limited to Oncology, Internal Medicine, Dermatology, Cardiology, and Emergency and Critical Care. The Oncology department collaborates with the doctors at the UF Health Cancer Center (UFHCC) as part of a Comparative Oncology Initiative, which has many ongoing canine and feline trials focusing on immunotherapy. **RESULTS/ANTICIPATED RESULTS:** As of August 2023, there are 60 clinical trials actively recruiting and enrolling patients at the UF CVM. 57% of these trials are interventional studies, while the other 43% are observational studies. The UFHCC Comparative Oncology Initiative has successfully completed one clinical trial focusing on canine gliomas; has 4 clinical trials that are actively recruiting patients, and 6 trials that are opening for enrollment in the near future. These studies focus on osteosarcoma, melanoma, and squamous cell carcinoma. It is anticipated that with continued successful collaborations, more clinical trials will be possible, and new treatment options will become available for not only veterinary patients but human patients as well. **DISCUSSION/SIGNIFICANCE:** Clinical and translational research is an important part of veterinary medicine to further patient care. Due to ongoing collaborative efforts, not only veterinary patients but also human

patients will benefit from the research being conducted at the UF CVM.

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### **Implementation of COPD Clinical Practice Guidelines with Use of Telehealth**

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**OBJECTIVES/GOALS:** Studies to improve uptake of Chronic Obstructive Pulmonary Disease Clinical Practice Guidelines (COPD CPG) have yielded inconsistent results. We hypothesized that using implementation science would facilitate rigorous site 'diagnosis', and promote effective contextual tailoring of COPD CPG, while piloting the use of telehealth for this. **METHODS/STUDY POPULATION:** The study was conducted in two Veterans Affairs primary care clinics located in a small sized city. A detailed formative evaluation was conducted using key informant interviews (with VA staff and veterans with COPD who received care at this location) and quantitative data. Multidisciplinary stakeholder group was engaged and strategies to address the determinants identified through the previous step were identified. Telehealth was strongly encouraged as the primary modality for implementing the COPD CPG and we are collecting pilot data on this. Tele-Facilitation, used as the meta-strategy was employed in conjunction with other strategies such as develop/distribute educational materials, tailor strategies, change record systems and revise professional roles. **RESULTS/ANTICIPATED RESULTS:** Primary Care at the VA is provided by Patient Aligned Care Teams (PACT-teams), where each team consists of multiple health professionals to provide collaborative care to the patient. Discussions with the multidisciplinary stakeholder team suggested that any implementation effort primarily focused on physician and nursing efforts was unlikely to succeed due to competing demands. A pharmacy-centric model that allowed for the PACT-team clinical pharmacist to address most of the COPD CPG (inhaler technique education/assessment, inhaler choice optimization, COPD-specific patient education, spirometry use, smoking and immunization) was developed and implemented with incorporation of telehealth (video visits and telephone). We will present pilot implementation outcomes using RE-AIM framework elements. **DISCUSSION/SIGNIFICANCE:** This use of implementation science to implement COPD CPG and novel use of telehealth has enormous potential for impact. Increasing reach/adoption by targeting primary care practices can help permeate quality care to the underserved population. This data will allow us to explore generalizability through wider scale implementation studies.

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### **The Effect of a Culturally-tailored and Theory-based Resistance Exercise Intervention on Motivation, Self-Regulation, and Adherence in Young Black Women\***

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**OBJECTIVES/GOALS:** Black women participate in the least amount of physical activity in the U.S., and determining methods to increase