(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 ± 2.01%) and placebo (2.24 ± 1.69%) conditions ($p = 0.48$; $d = 0.35$). Furthermore, peak RH did not differ between the nitrate-rich (1503 ± 443 ml/min) and placebo (1762 ± 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.

358

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

Michael Campbell, Mokshitkumar Patel, Shuo Xiao, Xia Wen and Lauren M. Aleksunes
Rutgers University

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 μM 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 10μM cyclosporin A, a general OATP inhibitor for 1 hr, and then incubated with 1μM 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 10μM. 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.

362

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

Dusti Jones, Lindsey N. Potter, Cho Y. Lam and David W. Wetter
University of Utah

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.

363

A CRISPR/dCas9 Epigenetic Therapuetic Approach for CASK-Related MICPCH*

Casiana Gonzalez, Julian Halmai and Kyle Fink
University of California, Davis

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

Downloaded from https://www.cambridge.org/core. IP address: 3.141.25.201, on 14 Oct 2024 at 23:27:57, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms.
https://doi.org/10.1017/cts.2024.321