STUDY TITLE: Modifiable predictors of neural vulnerabilities for obesity

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Study Protocol

Objective and Background: The purpose of the trial is to examine activation in a priori selected brain regions associated with reward processing during exposure to sips of milkshake among young adults. Explicating patterns of reward processing for appetizing food stimuli is important for understanding neural underpinnings of obesity risk.

Design: This observational trial involved a functional magnetic resonance imaging (fMRI) task which was completed by all trial participants. The participants were young adults (at least age 19 years) who were either 1) participants in an ongoing longitudinal study of development and health (the Preschool Problem-Solving Study at the University of Nebraska-Lincoln); or 2) undergraduate students who lived in the geographic area of the study (Lancaster County, NE) for at least part of high school. Exclusion criteria included any contraindications for MRI including metal in body, pregnancy, braces, non-removal piercings, or hair extensions. Potential participants gave written informed consent and were screened for exclusion criteria to ensure safety. Participants then completed the fMRI task (see task description below) with a research assistant and MRI technologist. All participants completed the same task (i.e., all were assigned to the same arm of this single-arm trial) to produce descriptive results.

Methods: Participants completed a well-established fMRI task (i.e., the “milkshake task”) intended to measure sensitivity to appetizing stimuli via activation in brain regions associated with reward processing. In the task, participants are shown a cue (a picture of either a glass of milkshake or water for 5 seconds) to signal impending taste of the respective beverage, followed by a jitter of 2-7 seconds during which the screen is blank. Next, 0.7cc of the fluid (milkshake or tasteless solution) is delivered over 5 seconds, followed by a jitter of 2-7 seconds. A swallow cue is then presented for 5 seconds. This trial format is repeated throughout the task with an equal number of trials presenting milkshake and tasteless solution. Brain activation, as represented by blood oxygen level-dependent (BOLD) signal, is assessed via a 3T MRI scanner, both in anticipation of the taste delivery (i.e., cue onset) and to the delivery of fluid (delivery onset).

Statistical Analysis Plan

BOLD activity will be modeled using hemodynamic response functions at the onset times for the cue and delivery for both milkshake and tasteless trials. Additionally, motion parameters, baseline and drift estimates will be included as regressors of no interest. Brain activity for *cue* (anticipation) will be calculated by extracting the beta values for all milkshake cue trials versus all tasteless solution cue trials from bilateral 5mm radius spheres located in the following locations based on previous literature and theory: caudate (MNI coordinates +/-9,13,0), putamen (MNI coordinates +/-24,0,3), insula (MNI coordinates +/-33,23,3). Brain activity for *delivery* will be calculated in the same way following the delivery of milkshake or tasteless solution across all trials.

The above procedures will result in two primary sets of outcome scores for each individual participant: 1) a difference score between milkshake and tasteless *cues* for the caudate, putamen, and insula, representing activation in each area for the milkshake cue versus the tasteless cue; and 2) a difference score between milkshake and tasteless *delivery* for the

caudate, putamen, and insula, representing activation in each area for milkshake delivery versus tasteless solution delivery.

Finally, the individual-level scores for cue and delivery will be used to calculate sample-level descriptive summary statistics (e.g., mean, standard deviation) for each outcome variable. Because there is only a single-arm in this observational trial (i.e., all participants completed the same task with no random assignment to different conditions), the main outcome variables for the trial will be descriptive and no inferential statistical tests comparing groups can be conducted.