Cover Page

Official Title: **A Multimodel Examination of Bromocriptine on Homeostatic and Hedonic Mechanisms of Food Intake in Individuals at High Risk for Type 2 Diabetes**

NCT Number: **NCT05405244**

Document Date: **June 9, 2022**

Statistical Analysis Plan

A. Sample Size and Power

We will recruit and screen 180, 18-35 year old overweight/obese individuals in order to enroll 50 participants with and without the presence of the DRD2 TaqIA A1 allele, and at high risk for T2DM by virtue of HbA1c. The n=42 represents the number of participants who will provide usable data and accounts for possible attrition and negative side effects of the bromocriptine (based on previous repeated measures fMRI studies and reports of bromocriptine, expected attrition is 4%-7%). In evaluating our inclusion/exclusion criteria (see below) conservatively we anticipate 180 participants per year will be eligible for the present investigation. Of those, based on previous work, 36 are expected to have one A1 variant of the TaqIA polymorphism and 144 to have A2/A2 (per each recruitment year). Therefore, over the three-year duration of the present study, we will have ample opportunity to meet our recruitment targets (Y1: n= 17; Y2: n= 18; Y3: n= 15). Power analyses were conducted using fMRIpower (for fMRI contrasts described in Aim 1b) and G*Power (for lab measures) to determine sample size based on previous work (25). Sample sizes were determined by the between-group (TaqIA allele status; Aim 2) difference, which is the least sensitive of the proposed tests. Based on a small to medium sized effect (r = .30), we determined that a similarly sized strategy-by-assignment interaction would be detectable with 0.8 power with at least n=21 per group, yielding a total sample size of 42. To account for the small possibility of complications with blood draws, excessive movement in the scanner, side effects from the medication or other protocol deviations we plan on recruiting a total of 50 (25 per cell).

B. Analysis Plan

1. Primary and Secondary Outcomes

Behavioral data analysis (food intake + hedonic ratings of food). Continuous variables will be summarized by descriptive statistics (e.g., mean, SD, range, median), examined via graphical analyses (e.g. histogram, Q-Q plot) and analyzed to assess the normality of the data. Data transformation of continuous variables will be used as necessary. Model assumptions and potential outliers will be analyzed with various modeling diagnostics and plotting techniques. Visual analog scale (VAS) measures will be analyzed using PROC MIXED implemented in SAS (Version 9.4, Cary, NC). VAS scales for milkshake, snack food pleasure and desire, ad lib intake of those foods as well as changes in hunger (fasted to fed) will be entered into 2x2 repeated measures mixed models including drug-by-gene interactions which addresses Aim 1a and Aim 2. Control variables of no interest to be included are BMI, gender, visit number and nausea (after scan assessments only). In addition to main effects of the drug and the TaqIA allele status, interactions with $p < 0.10$ will be probed with least squared mean comparisons of a priori contrasts of interest including tests of the: 1) impact of TaqIA allele status with bromocriptine, 2) impact of TaqIA allele status without bromocriptine 3) impact of bromocriptine on those with the TaqIA A2/A2 variant, and 4) impact of bromocriptine on those with at least one TaqIA A1 variant.

fMRI data preprocessing: Neuroimaging data will be preprocessed using the fMRIPrep pipeline [45]. DICOMS will be converted to the Brain Imaging Data Structure (BIDS file structure) [46], then preprocessed using fMRIPrep. fMRIPrep preprocessing includes skull stripping using Advanced Normalization Tools (ANTs); susceptibility distortion correction using field maps; tissue segmentation using FSL's Automated Segmentation Tool (FAST); and spatial

normalization to Montreal Neurological Institute (MNI) 152-Asymmetrical space using ANTs' registration option. Functional data will be co-registered to anatomical data using FSL's FMRIB's Linear Image Registration Tool (FLIRT) with boundary-based registration. Functional data will additionally be motion corrected using FSL's MCFLIRT. In FSL, final preprocessing will include spatial smoothing using a 5mm full width half maximum isotropic Gaussian kernel, high-pass filtering, and adjusting for nuisance regressors, including the 6 motion parameters, the derivatives of each, and high motion time points (FD>0.9mm). A functional run for a subject will be excluded if >25% of total volumes are flagged as high motion points.

fMRI primary analyses: Neuroimaging analyses will be primarily completed in FSL (FMRIB Software Library,www.fmrib.ox.ac.uk/fsl). Within-run (level 1), within-subject (level 2) and group-level (level 3) analyses will be carried out in FSL's FMRI Expert Analysis Tool (FEAT). At the 1st (within-run) level, models will assess brain response to the following contrasts of interest: effect of milkshake cue (milkshake cue > h2o cue) and effect of milkshake taste (milkshake taste > h2o taste). Inverse contrasts (h2o cue > milkshake cue; h2o taste > milkshake taste) will also be assessed.

At the 2nd level analyses, to test Aims 1 and 2, milkshake cue > h2o cue and milkshake taste > h2o taste contrasts will be carried forward to a fixed effects within-subject t-test estimating individual-level differences as a function of bromocriptine, i.e., active drug (bromocriptine) > placebo. Bilateral striatum will be used as the a priori region of interest (ROI). Binary masks of caudate, putamen and ventral striatum will be included in the level 2 within-subject models to extract parameter estimates for the contrasts of interest only within those regions. Extracted parameter estimates will be averaged across the three regions resulting in a single striatal parameter estimate value per contrast per subject.

For group-level analyses, striatal parameter estimates will be entered in 2x2 repeated measures mixed models including drug-by-gene interactions. As there are two scans, pre-scan covariates will likely vary between the two scans, as such pre-scan thirst and milkshake pleasantness ratings from both visits will be included as time-varying covariates. Models will also include visit order, BMI, and gender as covariates.

2. Exploratory Outcomes

Appetitive hormone analysis: Participants will have three blood draws per visit. For each visit, the first blood draw (fasting) will occur at the start of the visit prior to drug administration. Second blood draw will occur 180min after drug administration (and 30min after a fixed milkshake consumption). Third blood draw will occur 210min after drug administration (and 60min after a fixed milkshake consumption). Blood samples for satiety hormones will be assayed using Bio-Plex Pro Human Diabetes 7-plex Assay (Bio-rad, Hercules, CA). This kit analyzes adiponectin, amylin, and GLP-1. Additionally, a custom Human Cancer Panel 1 with only prolactin will be used to analyze prolactin concentrations (Bio-rad, Hercules, CA) using a Millipore Luminex (Darmstadt, Germany). Using the 3 timepoints for the blood draws, we will create the area under the curve (AUC) for each appetitive hormone (prolactin, adiponectin, amylin, GLP-1, insulin) for each visit. AUC values will be entered into 2x2 repeated measures mixed models including drug-by-gene interactions. Control variables of no interest to be included are BMI, gender, visit number, and baseline (fasting) hormone levels.

Behavioral response inhibition analysis: Reaction time, % commission errors and % omission errors from the Stop Signal Task will be used as a behavioral measure of inhibition. The three outcome variables will be entered into 2x2 repeated measures mixed models including drug-by-gene interactions. Control variables of no interest to be included are BMI, gender, visit number, and pre-task nausea ratings.