PART B
STUDY DESCRIPTION

TITLE OF PROTOCOL	Neurological impacts of artificial sweeteners in the context of diet sodas
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B1. PURPOSE OF PROTOCOL

Specific Aim. To determine whether and how artificial sweeteners in diet sodas impact appetite in the brain using fMRI to food cues (highly vs. less desirable) as compared to regular soda or carbonated water. Also to confirm with neurocognitive testing whether the potential networks involved in eating behaviors (e.g. cognitive control, working memory) may be impacted by artificial sweeteners.
Hypothesis 1: Diet soda will show no differential brain activations as compared to carbonated water.
Hypothesis 2: Changes in brain activations will correspond to ratings of appetite.
Hypothesis 3: Diet soda will show no changes in neurocognitive tasks which may impact eating behaviors (e.g. cognitive control, working memory).

B2. SIGNIFICANCE AND BACKGROUND FOR THE STUDY

Obesity is a growing and significant problem in the United States where approximately two-thirds of adults are overweight (body mass index- BMI 25-29.9) or obese (BMI ≥ 30). In turn, obesity, and especially central obesity, leads to or exacerbates many health problems, accounting for approximately 200,000 deaths annually from diabetes (DM), hypertension (HTN), cardiovascular disease (CVD), liver and renal disease, and cancer (1). Worldwide, obesity and associated metabolic morbidity are alarming problems that are extremely challenging to treat (2). Understanding the dietary and other components which may lead to or prevent obesity are critical to developing clinical treatments and nutritional interventions.

Artificial sweeteners, particularly in the context of diet sodas, have come under fire for their relationship with obesity in the context of observational and uncontrolled studies (3-6). This led to the hypothesis that artificial sweeteners used in these beverages could cause an insulin response to the sweet taste which results in the consumption of additional calories through other food sources. Indeed, some studies in rodents found an insulin spike in response to artificial sweeteners (7, 8). However, another study observed that only artificial sweeteners with a bitter taste (acesulfame-K, stevioside) increased insulin secretion whereas aspartame, which does not have the bitter taste, did not have any effects on insulin secretion in rodents (9). Regardless, rodents are inherently different than humans, who have a more complex control of appetite and digestion.

There are a few studies which have examined the effects of diet sodas or artificial sweeteners on insulin and other gut hormone responses. In 7 healthy humans, Ma et al. (10) found no difference between sucralose and saline in terms of glucose, insulin, glucagon-like peptide 1 (GLP-1), or gastric inhibitory peptide (GIP). Similarly, a study of 12 healthy volunteers found no changes in glucose, insulin, GLP-1, peptide YY (PYY), or ghrelin with aspartame, acesulfame K, sucralose, or 2-deoxy-d-glucose, which is similar in structure to glucose, suggesting that the secretion of these gastrointestinal peptides does not rely on sweet taste in humans (11). However, another study in 22 healthy volunteers demonstrated

an increase in GLP-1 (which may actually decrease appetite), but not insulin or glucose, secretion with diet soda (12). Importantly, several studies have shown no changes in appetite and/or caloric consumption after diet soda or artificial sweetener intake in humans (for a thorough review, see Bellisle & Drewnowski (13)), suggesting that these findings of lack of changes in gut hormones related to satiety and energy homeostasis are true. Thus, the evidence suggests that although there may be changes in insulin and other gut hormones secretion due to artificial sweeteners in rodents, this may not hold in human studies. However, critically, these findings may also be impacted by obesity and should be studied in an obese population.

Furthermore, when considering appetite in humans, it is particularly critical to consider the brain (14). Although these studies have focused on the gut, the brain is an even more critical area to examine, as changes in neural activity can have impacts on appetite that would not be captured through gut hormone analysis. Although no studies have examined whether diet sodas or artificial sweeteners may alter the way the brain perceives food, Smeets et al. (15) found that although glucose resulted in a significant 'resting' decrease in the hypothalamus, artificial sweeteners aspartame and maltodextrin did not result in any signal changes in the hypothalamus. However, no other brain areas were analyzed and no food cues were used, so this could only be applied to general activation changes. Another study examined the effects of carbonation on taste of sucrose vs. artificial sweeteners and found that only sucrose significantly changed 'resting' brain activity (16). Again, this did not use food cues or examine appetite-related signals. Diet sodas may have important effects on appetite, particularly as compared to regular sodas, which cannot be captured in these previous studies.

Thus, we propose a randomized, controlled, cross-over study to examine the effects of artificial sweeteners in the context of diet soda (Diet Coke- aspartame) as compared to regular soda (Coke) and carbonated water (to control for effects of carbonation, e.g. fullness), on appetite signals in the brain using functional magnetic resonance imaging (fMRI). We will connect these findings with ratings of appetite. These results will critically provide pilot data to determine whether and how artificial sweeteners/diet sodas may impact appetite in the brain.

B3. DESCRIPTION OF RESEARCH PROTOCOL

A. Study Design – Overview, Methods, Procedures

5 obese (body mass index, BMI ≥ 30kg/m^2) subjects will participate in this randomized, controlled, cross-over pilot study to examine the effects of artificial sweeteners (aspartame) in the context of sodas as compared to sugar-sweetened soda and carbonated water. To be able to match for other ingredients somewhat closely, participants will consume Diet Coke (aspartame), Coke (regular sugar-sweetened beverage) or carbonated water.

Participants will be screened over the phone to ensure they meet inclusion/exclusion criteria before being invited for the study visits. They will be asked to abstain from consumption of any carbonated beverage products for 2 weeks before beginning the study. Each participant will have three study visits in a randomized order and separated by at least a week during which they will receive each of the three options (aspartame, glucose, or none). All other aspects of each visit will be identical, and all participants will have all three visits/conditions in a randomized order. At each study visit, the participant will come to the MRI Research Center (Ansin 3rd floor) at Beth Israel Deaconess Medical Center (BIDMC) to consume the beverage which they have been randomized to receive at that visit followed by an fMRI scan during which they will view highly desirable, less desirable, and non-food cues using a block design (see Methods below for more details). Before and after the fMRI scan, they will complete visual analog scales to rate hunger and fullness. They will finish by having a battery of neurocognitive tests.

MRI protocol: Participants will view food and non-food items within a 3 Tesla MRI scanner. Using this fMRI data, we will be able to determine which neuroanatomical areas are most influenced by diet soda, regular soda or carbonated water to food cues. Scanning will be carried out using a protocol similar to that previously described (17). First, in each of the scanning sessions, a T1-weighted MPRAGE (Magnetization Prepared Rapid Gradient Echo) structural MR image will be acquired. Next, gradient-echo T2-weighted echo planar images depicting blood oxygenation level-dependent (BOLD) contrast will be acquired from non-contiguous near axial planes. Subjects will view pictures of high fat or high calorie (highly desirable) foods, low fat or low calorie (less desirable) foods, and control (non-food) stimuli. These will be presented with a block design (each block consisting of either 5 successive high fat or low fat food or 5 successive non-food pictures). E-Prime software controls stimulus presentation. Images will be presented in blocks, and each block will be presented in a counterbalanced order and interspersed with periods of visual fixation. All food and non-food images will be matched for visual properties.

Visual Analog Scales (VAS): VAS are used to measure subjective feelings of hunger and fullness/satiety before and after the beverage (diet soda, regular soda, or carbonated water) administration and fMRI. Participants mark along a line (as a scale of 1-100) how hungry or full they feel as well as how pleasant it would be to eat and how much volume they feel they could eat. VAS are analyzed and anticipated to represent changes in hunger.

Cognitive Testing: Cognitive testing will be performed on the CANTAB machine, which provides verified and scientifically proven tasks for easy and controlled administration to test subjects. We will include a number of important tasks, including:

- *Stop-Signal Task (SST):* The SST is a classic behavioral inhibition test, which uses a staircase procedure to generate an estimate of the stop signal reaction time, which measures an individual's ability to inhibit a prepotent response. Thus, this provides a measure of inhibitory control.
- *Intra-Extra Dimensional Set Shift (IED):* The IED is a test of rule acquisition and reversal. It features visual discrimination and attentional set formation, as well as maintenance, shifting and flexibility of attention.
- *Verbal Recognition Memory (VRM):* The VRM test assesses immediate and delayed memory of verbal information under free recall and forced choice recognition conditions.
- *Spatial Span (SSP)*: The SSP assesses general working memory capacity.
- *Spatial Working Memory (SWM):* The SWM provides a measure of spatial working memory capacity as participants are required to remember where items have appeared.

B. Statistical Considerations

Sample Size Justification: This is a pilot study, and the data generated herein will be used to determine sample sizes needed for future, larger studies.

Statistical Analysis: We will use analysis of variance (ANOVA) to compare the changes between appetite-related hormone levels (insulin, glucose, adiponectin, leptin, GIP, GLP-1, and ghrelin) and hunger levels (VAS) during each beverage condition. We will also perform multivariate analysis by fitting a mixed model with a random subject component permitting correlation within subjects and non-constant variability, and controlling for study sequence and fat mass.

MRI Analysis: Data will be analyzed with SPM12 (Statistical Parametric Mapping, http://www.fil.ion.ucl.ac.uk/spm/). Images will be re-aligned, co-registered to the patients' own structural images, then spatially normalized to a standard template and spatially smoothed with a Gaussian kernel (6 mm at full width half-maximum). The time series in each session will be high-pass filtered (with cut-off frequency 1/120 Hz) and serial autocorrelations will be performed. Then, we will build a design matrix where we will map the timing of the image presentation to the fMRI images. Finally, we will use the data from each individual subject in group analyses. For example, we will perform the following: (a) flexible factorial analyses examining whether the difference in neural activation to food minus non-food stimuli varies as a function of group (i.e., diet soda, regular soda, and carbonated water); (b) analyses examining the moderating influences of group/treatment upon the relation between self-reported ratings of food and differences in neural activation to food and non-food stimuli; and (c) mediational analyses between patients, blood hormone levels, and differences in brain responses to food versus non-food cues.

C. Subject Selection

Inclusion Criteria

Men and women who are 18-65 years old, with a BMI >30kg/m2. Control: The cross-over design employed for this study allows study participants to function as their own control.

Exclusion Criteria

Unable or unwilling to participate in the study for any reason
Metal in the body or other safety concerns which makes patient unable to have an MRI
Pregnant women will be excluded due to the difficulty of measuring accurate BMI.

B4. POSSIBLE BENEFITS

Obesity and diabetes have reached a prevalence of epidemic proportions in the United States of America and are a growing problem world-wide. Obesity is associated with increased incidence of heart disease, stroke, type 2 diabetes, and certain types of cancer, some of the leading causes of preventable death. Diabetes is, also, associated with several serious or life-threatening conditions and diseases such as retinopathy, neuropathy, renal disease, cardiovascular disease and many more. Participation may help others in the future as a result of knowledge gained from the research.

Thus, while these studies do involve minimal risks to subjects, we feel that the potential benefit derived from these studies significantly outweighs the relatively low-magnitude of risks associated with this research, since these studies will contribute to medical knowledge and potential development of new recommendations for this condition.

While the proposed study is unlikely to benefit participants directly, the findings from this study will provide information on the impacts of diet sodas to alter eating behaviors in humans. As there are potential links between obesity and diet sodas, this study can provide potential information about mechanisms of these links.

B5. POSSIBLE RISKS AND ANALYSIS OF RISK/BENEFIT RATIO

Inclusion and Exclusion criteria have been defined so that benefit is maximized and risk is minimized. We have utilized similar criteria in our past studies to date and have significant experience with similar studies. To protect patients from psychological risks, they have the right to refuse answering any questions that they do not feel comfortable responding to.

fMRI: There may be risks or discomforts associated with performing an MRI scan, such as fear of being in a tight space. Certain metals are dangerous in an MRI, such as a pacemaker, a metal heart valve, an infusion pump, or a brain aneurysm clip, though you will be carefully screened to make sure that these are not present inside you. If a patient is uncomfortable during the MRI, they have the choice to come out of the scanner. All participants will be screened with a safety questionnaire prior to entering the fMRI area. They will be screened for any metallic objects or implants, eyeliner/tattoos, prostheses, shrapnel, and any medical conditions that may be contraindicated for fMRI imaging.

Neurocognitive testing: There are no anticipated risks or discomforts associated with testing. In order to minimize fatigue and stress, testing sessions will be kept as short as possible and subjects will be allowed frequent breaks, after each test.

Visual analog scales: No risks are associated with these hunger-related scales.

B6. RECRUITMENT AND CONSENT PROCEDURES

Recruitment

Subjects will be recruited without regard for racial, social, economic, or other status. We will submit all advertisements used for this study to the Institutional Review Board (IRB) for review and will obtain approval prior to use. We will advertise this study by posting flyers and posters in clinics and throughout the Boston and/or Greater Boston area and by publishing newspaper advertisements locally. All publicity materials generated for the study will include the contact information for a study physician so that potential subjects can obtain information about the study. Potential subjects will be pre-screened over the phone and if it seems as though a subject may qualify, he/she will be invited to schedule a screening visit.

Phone screening

The phone screen will be conducted as per the questions outlined in the submitted phone screening form. Prior to staff going over the phone screen form with the patient, they will be instructed to use something along the lines of the below script:

"Thank you for your interest in the study. First, I will go over some basic information about the study, and then, if you are still interested, I will ask you some questions to see if you might qualify.

"This study is interested in looking at how artificial sweeteners in the context of diet sodas may alter the way your brain thinks about food. Diet sodas have been associated with obesity in some studies, and we want to investigate this potential link further.

"We will first go through a phone screen of questions about your medical history, and then if everything looks good, we will bring you in for your first visit. At this visit, I will go over the study in depth and have you signed a consent form if you are still interested. After that, you will drink either diet soda, regular soda, or carbonated water and then have an MRI scan which will take pictures of your brain while you look at food and non-food images. You will then do some cognitive testing, which are like computer games. You will then come back two more times to repeat the visit with the two beverages you did not have before (either diet soda, regular soda, or carbonated water).

"Do you have any questions for me now? Are you still interested in participating in the study?

"I am going to ask you some detailed questions about your medical history to see if you might qualify for the study. You may stop me at any time. Do I have your permission to ask you these questions about your medical history?"

Consent

Subjects will meet with investigators in a secure room at the MRI Research Center where the purpose of the study, the procedures, risks, benefits, study reimbursement, and other details of the protocol will be explained to the subject. Subjects will be given a copy of the IRB-approved informed consent

forms to review, and they will have the opportunity to ask the study investigator questions about the protocol. After all questions by the subject have been answered, written informed consents will be obtained from the subject by a study investigator in the presence of a witness. The investigator will inform the subject that they have the alternative not to participate in the study and the right to withdraw from the study at any time. The investigator also has the right to terminate the study at any time should they determine that the subject no longer qualifies to take part, or if it would be dangerous for them to continue, or if they do not follow study procedures as directed by the investigators. Copies of the consent forms will be provided to the subject for his/her records.

B7. STUDY LOCATION

Privacy
The privacy of research participants will be protected at all times in accordance with BIDMC policies throughout all phases of the study.

Physical Setting
Subjects will be consented inside a private room at the MRI Research Center with the door closed to allow for privacy. Subject visits will take place at the Beth Israel Deaconess Medical Center MRI Research Center.

B8. DATA SECURITY

Protection of Subject Privacy
During this study, MRI, questionnaire (VAS), and neurocognitive testing data will be collected. Data will be kept in strict confidence. Confidentiality will be assured by use of identification codes, which will appear on MRI, VAS, and neurocognitive testing. All data will be stored in a secured, locked room.

Database Protection
The database will be secured with password protection. Analysis will be done with coded information, which is entered into the database under those identification codes. Any data saved electronically will be behind the BIDMC firewall, on password protected computers/drives.

B9 Multi-Site Studies

This is NOT a multisite study.

Is the BIDMC the coordinating site? ☐ Yes ☐ No

Is the BIDMC PI the lead investigator of the multi-site study? ☐ Yes ☐ No

B10 Dissemination of Research Results

All subjects will be thanked for their participation in the study. Study data can be requested by subjects to have for their own records. Study data may be published in a brief report as a pilot in a peer-reviewed journal for future use in future study design and/or for patients to see.

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