Clinical Research Protocol
National Institute of Diabetes and Digestive and Kidney Diseases

DATE: September 13, 2021 **PROTOCOL NUMBER:** 18-DK-0132
VERSION: 1.10 (I)
IND NAME/NUMBER: [F-18] Fallypride (#70,046), [C-11] Raclopride (#54,135)
IND SPONSOR: NIH Clinical Center (CC)
TITLE: Brain Dopamine Function in Human Obesity

SHORT TITLE: Dopamine and Obesity

IDENTIFYING WORDS: Energy Expenditure, Neurocognitive Function, Obesity, Dopamine Response

TYPE OF PROTOCOL: Natural History – Disease Progression/Physiology

ESTIMATED DURATION OF STUDY: 3 years

START DATE: June 2018 **END DATE:** December 2022

PRINCIPAL INVESTIGATOR:

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NUMBER AND TYPE OF PATIENTS:

	Number	**Sex**	**Age Range**
Volunteers	Accrual ceiling = 100	Male and female	18 – 45 years

PROJECT USES IONIZING RADIATION:
Research indicated: Yes RSC Approval Number: 2677 Expiration Date: 6/12/21

OFF-SITE PROJECT: No

MULTI-INSTITUTIONAL PROJECT : No

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Précis

Evidence from neuroimaging studies indicates that aberrant functionality in brain regions that support reward processing and habit formation may be related to an individual's eating behavior and obesity propensity. In particular, our previous research found that increased dopamine D2 receptor binding potential (D2BP) in the dorsal and lateral striatum was positively related to opportunistic eating behaviors, body fat, and body mass index (BMI). However, our findings were contrary to highly-cited previous reports of D2BP correlating with BMI in the opposite direction. The primary aim of this study is to elucidate the reasons for the conflicting results that used somewhat different methodologies.

Specifically, our previous study used positron emission tomography (PET) to measure D2BP using the dopamine D2 receptor antagonist radioligand [^{18}F]fallypride following a period of dietary stabilization and 3 hours after a standardized breakfast. Reports finding correlations between D2BP and BMI in the opposite direction have typically investigated subjects with higher BMI using the D2 receptor antagonist radioligand [^{11}C]raclopride. Furthermore, previous studies were typically conducted in the fasted state, but the subjects' prior food intake was not well-controlled. The present study will attempt to resolve the controversy by measuring D2BP using both [^{18}F]fallypride and [^{11}C]raclopride in 39 adults, 13 within each of three BMI strata to represent a large BMI range, under controlled overnight fasting conditions following a period of dietary stabilization. The primary aims are to estimate the mathematical relationship between striatal D2BP and BMI and determine the within-subject correlations of D2BP derived from [^{18}F]fallypride and [^{11}C]raclopride.

1. Introduction and Objectives

1.1 Influence of feeding and adiposity on brain reward, motivation, and habit circuitry

Orchestrated by the brain's reward system, the drive to consume food is a primitive instinct, which ensures that an organism maintains sufficient energy stores for survival and reproduction [1]. It has been hypothesized that obesity arises, in part, from alterations to central dopaminergic pathways, which may affect reward sensitivity and reinforcement learning as it relates to food consumption. Support for this hypothesis has emerged from positron emission tomography (PET) studies of obesity, where seminal work identified a negative association between body mass index (BMI) and striatal dopamine (DA) D2 receptor binding potential (D2BP) [2]. However, the precise relationship between BMI and D2BP remains inconclusive, as negative [3], positive [4], and null associations [5] have been reported. Moreover, confounding factors, such as subject age [6, 7], BMI range, preceding diet, and the pharmacological kinetics of various PET radioligands, may obfuscate results. ***The present study is designed to address these various confounders and provide more definitive data on the relationship between BMI and D2BP.***

In a recent review, Horstmann et al. [8] theorized that the discrepant associations between striatal D2BP and BMI may have been due to a nonlinear relationship between BMI and D2BP such that D2BP increases from low levels in the normal BMI range, reaching a maximum D2BP at moderate levels of obesity, but then decreases as BMI further increases (**Figure 1**).

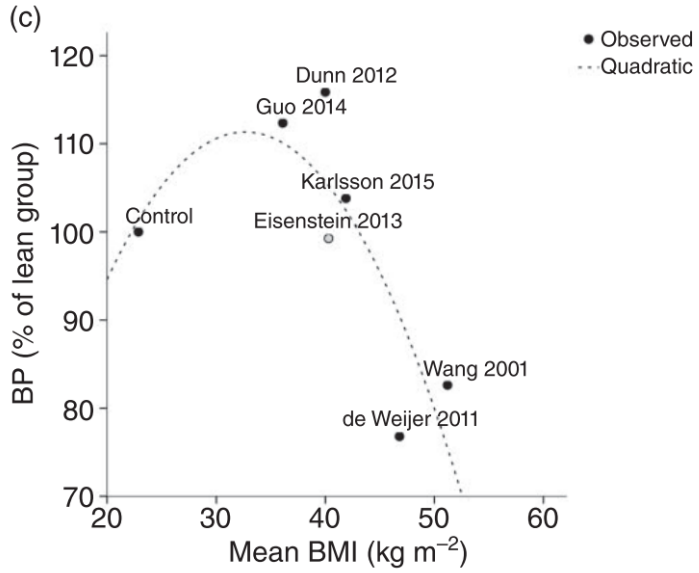

Figure 1. Theoretical quadradic relationship between BMI and D2BP hypothesized by Horstmann et al. [8].

The present study is designed to test this hypothesis in overnight fasted subjects after a period of dietary stabilization. The purpose of dietary stabilization is because metabolism of certain macronutrients, notably dietary carbohydrate and fat, augments endogenous DA levels in the brain, which may in turn affect PET estimates of D2BP. For example, post-ingestive signaling from dietary carbohydrate can induce central DA efflux in rodents independently from taste stimulation

[9, 10]. Food restriction in obese rats can conversely increase DA D2 receptor availability and expression [11]. As such, inconsistencies in DA D2 receptor binding in human obesity may reflect variability in prior macronutrient consumption. Indeed, previous unpublished findings from our laboratory indicate that striatal D2BP is significantly decreased in obese adults following 4 days of a low-fat diet.

In addition to dietary variation, the pharmacological kinetics of a given radiotracer may impact the measurement of D2BP. For instance, quantification of receptor availability using the moderate-affinity DA D2 receptor antagonist [^{11}C]raclopride may less precise than estimates derived from the radiotracer [^{18}F]fallypride, a competitive DA D2/D3 receptor antagonist. Such discrepancies are thought to arise from noncompetitive interactions between [^{11}C]raclopride and D2-like receptors, which reduce radioligand binding under certain conditions [12]. Our preliminary work has compared the reliability of these radioligands by simulating interactions with DA D2 receptors under variable physiological conditions (e.g., tonic DA levels, receptor availability). These simulations indicated positive correlations ($r = 0.66 \pm 0.006$) between binding potential estimates of [^{18}F]fallypride and [^{11}C]raclopride in humans; however, this has yet to be examined in vivo.

This study will implement within-subject PET imaging to investigate the reliability of two radioligands, [^{18}F]fallypride and [^{11}C]raclopride, in determining DA D2 receptor availability in individuals stratified by BMI. We will also examine the effect of a palatable meal on striatal DA D2 receptor binding potential.

Hypothesis 1: Following dietary stabilization, the relationship between striatal D2BP and BMI is quadratic.

Hypothesis 2: Striatal D2BP determined by [^{11}C]raclopride and [^{18}F]fallypride will be moderately correlated ($r > 0.6$).

Hypothesis 3: Striatal D2BP will be significantly reduced following a palatable meal as determined by [^{11}C]raclopride.

1.2 Primary Aims

1. To determine if D2BP is related to BMI and whether there is a linear or quadratic relationship as previously hypothesized.
2. To determine correlations between D2BP, as measured by [^{18}F]fallypride and [^{11}C]raclopride time-activity curves.
3. To determine the effect of palatable meal consumption on D2BP in individuals with a wide BMI range. Binding potential estimates will be estimated within subjects using [^{11}C]raclopride.

1.3 Exploratory Aims

1. Investigate the associations between brain dopamine D2BP and neural bases of food perception and preference.
2. Investigate the metabolic and endocrine correlates of brain dopamine D2BP and neural bases of food perception and preference.
3. Investigate the behavioral correlates of brain dopamine D2BP such as free-living physical activity, ad libitum meal consumption, and body weight changes over a one year period.

4. Investigate the psychological correlates of brain dopamine D2BP as determined by various questionnaires and computer tasks.

With the exception of the neuropsychological procedures completed at screening visit, a number of procedures described in the following text are pertinent to exploratory study aims outlined above. We will make every effort to complete and collect all of these exploratory measures in order to generate hypotheses for future studies. However, for various reasons, it may not be feasible to collect each measure on every participant.

2. Study Design and Methods

This study will investigate adult volunteers, stratified by BMI who will be invited to participate in 2 PET scan sessions. Thirty-nine volunteers will be examined while admitted as inpatients to the Metabolic and Clinical Research Unit (MCRU) at the NIH Clinical Center. In advance of the first PET session, interested volunteers will be pre-screened via telephone, and those deemed potentially eligible for the study will be invited to the Clinical Center to provide informed consent, complete a medical evaluation, and complete cognitive testing. Subjects will be required to consume a standard diet provided by the NIH Clinical Center for up 5 days prior to the inpatient visit.

Upon completion of the inpatient portion of the study, participants will be asked to complete 13 days of outpatient monitoring of weight, activity and energy balance (via doubly labeled water). Finally, participants will be invited to complete a 1-year follow-up assessment approximately 12 months after the final scanning session. Subjects will be asked to complete physical activity and body weight monitoring during the 12 months between their inpatient visit and 1-year follow up outpatient visit. The NIH will provide commercial accelerometers and scales to the subjects to enable remote monitoring. Additionally, during this 12-month period, participants will be provided with research grade accelerometers for periodic 7-day actigraph assessments. Subjects will be invited back to the NIH for MRI data acquisition and body weight measurements at the one-year follow up mark.

Table 1 and **Figure 2** indicate the timeline for eligible volunteers who have consented to participating in PET scanning. Scan conditions (e.g., [^{18}F]fallypride, and [^{11}C]raclopride/'milkshake challenge') will be counterbalanced across subjects.

Table 1. Example PET study timeline, including outpatient screening, inpatient procedures, outpatient monitoring, and 1-year follow-up assessments. The order of [F^{18}]fallypride or [C^{11}]raclopride/ milkshake challenge san for the 2 inpatient scan days will be randomized to the extent possible given PET department scheduling constraints. Phase B of the study will occur as up to 11 contiguous inpatient days although to minimize volunteer burden, volunteers may opt to schedule Phase B as two or three separate two-day inpatient visits.

	Phase A	**Phase B**		**Phase C**	**Phase D**
	Visit 0 Screening (5 – 7 hours Outpatient)	**Stabilization diet (up to 5 days leading up to admission) Inpatient or Outpatient**	**Visit 1 (up to 6 days Inpatient)**	**Outpatient monitoring (13 days)**	**1-Year FU (8-hour visit + outpatient weight & activity monitoring)**
Subjects provide informed consent	X				
Questionnaires & Computer tasks	X		X		X
Blood sampling (screening and/or research collection)	X	X	X		X
Urinary drug test	X				
Daily Body weight	X		X	X	X
Physical health assessment	X				X
Body composition (e.g., DEXA, BodPod, BIS)			X		X
Metabolic cart (Resting energy expenditure)			X		X
Continuous glucose monitoring			X		
Activity monitoring (accelerometers)			X	X	X
Doubly labeled water sample collection				X	
PET scan ([F^{18}]fallypride)			X		
PET scan ([C^{11}]raclopride; milkshake challenge)			X X		
Brain MRI scan			X X		X

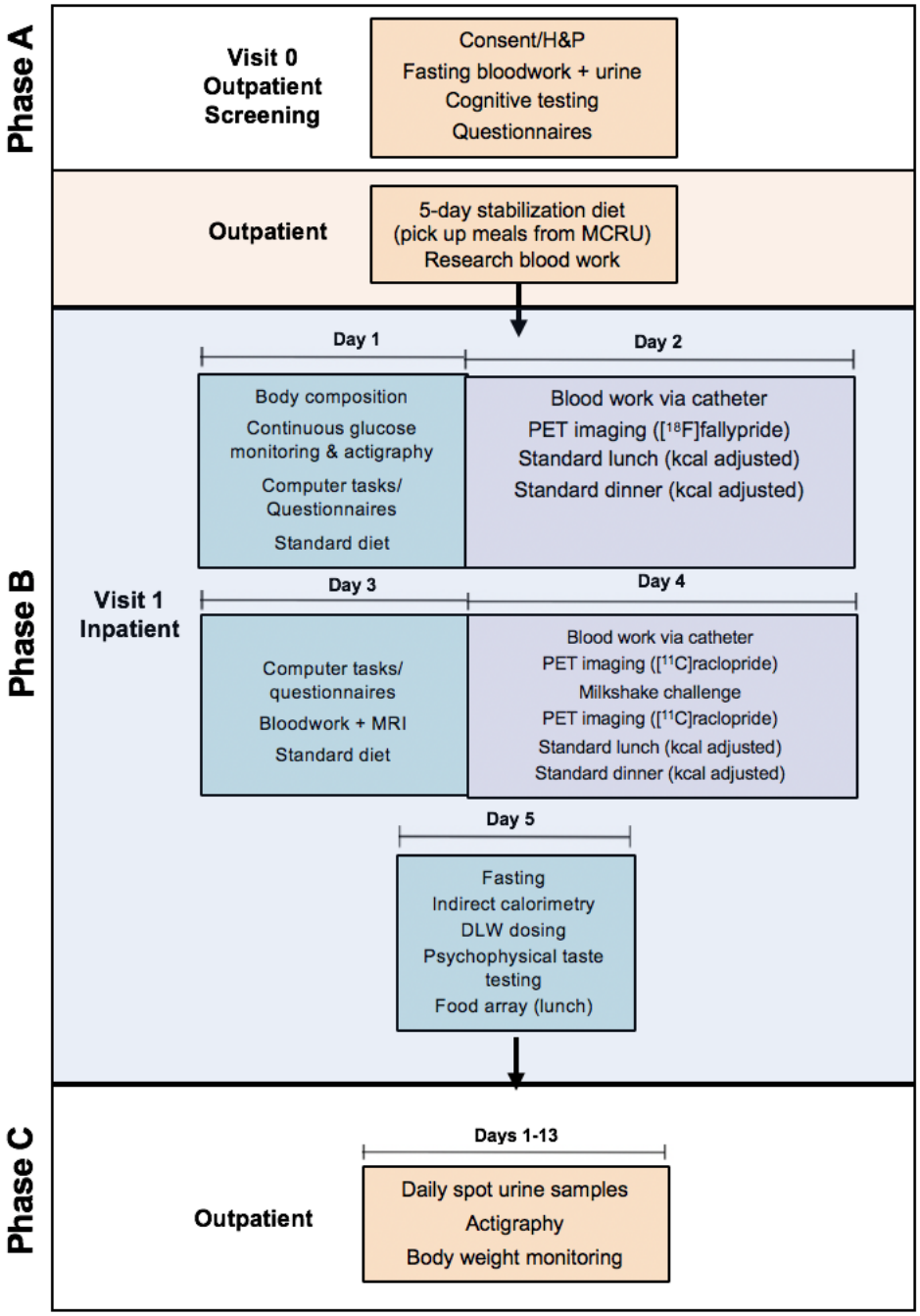

Figure 2. Example schedule of inpatient visits for PET scanning and outpatient portion for monitoring of energy balance. Note, for inpatient portion, the order of [F^{18}]fallypride or [C^{11}]raclopride will be randomized to the extent possible given PET department scheduling constraints.

2.1 Subjects

39 subjects will be recruited over a wide BMI range (18.5 kg/m^2 ≤ BMI) through the NIH Patient Recruitment and Public Liaison Office. NIH employees will be eligible to participate in this study. The research team will contact potential study volunteers to describe the study and perform pre-screening via telephone. The interested volunteer will be asked to verbally consent to participation in the telephone pre-screening. Telephone pre-screening will exclude interested individuals with a BMI <18.5 kg/m^2, those with previous bariatric surgery or other metabolic disorders, those with previous traumatic head injury or neurological disorder, those with food allergies (e.g., dairy, gluten) or those who require assistance to complete activities of daily living. Interested adults will also be asked questions related to eating behaviors, health history, and MRI compatibility. Potential volunteers meeting phone-screen criteria will be invited to schedule a screening visit at the Clinical Center and mailed a copy of the study consent form for advance review.

At the screening visit, potential volunteers will meet with members of the research team, review the study, and provide informed consent for the PET protocol outlined in **Table 1**. Volunteers will review the consent form while the researchers fully describe each component of the study. To the extent possible, portions of the screening visit may be completed via telehealth appointment. All subjects will be fully informed of the aims, nature, risks, and potential benefits of the study prior to giving written consent.

We plan to have an accrual ceiling of 100 subjects to account for those who withdraw from the study or are unable to complete the study. We will implement a "recruit-to-replace" strategy to ensure that early attrition does not compromise the study power of 39 subjects for Specific Aim 1. We will recruit 13 subjects in each of the following BMI strata: 18.5 kg/m^2 ≤ BMI < 25 kg/m^2, 25 kg/m^2 ≤ BMI < 35 kg/m^2, BMI ≥ 35 kg/m^2.

To achieve sufficient power to test Specific Aim 3 in an independent cohort of participants, we will recruit an additional maximum 21 subjects (7 maximum in each of 3 BMI strata), for a total of 60 enrolled participants max.

2.2 Inclusion and Exclusion Criteria

Inclusion criteria:

- Age 18-45 years, male and female
- Consent to undergoing PET scanning
- Body mass index (BMI) ≥ 18.5 kg/m^2
- Weight stable (less than ± 5% change in the past month)
- Written informed consent
- Estimated IQ ≥ 70, as determined by the NART (Scores below 70 are indicative of mental retardation; IQ has been related to alterations in brain structure and function that may confound neuroimaging measures. Failure to meet this eligibility criteria will be documented in the record and communicated to the potential participant as "ineligibility based on reading test results")

Exclusion criteria:

- Age 46 or greater (Age is a significant confound in the relationship between BMI and dopamine [6]. Dopamine binding has been shown to drastically decrease in the fifth decade of life [7].
- Body weight > 400 lbs. (weight limit of PET scanner)
- Weigh less than 80% of maximum lifetime weight
- BMI < 18.5 kg/m^2
- Past or present history of neurological or psychiatric disease (e.g., depression, anxiety, substance use disorder or psychosis), or eating disorders (e.g., anorexia nervosa, bulimia nervosa, or binge eating disorder), as determined by research team upon review of history/physical, Eating Disorder Examination-Questionnaire and Self-Rated Level 1 Cross-Cutting Symptom Measure.
- Blood pressure >140/90 mm Hg
- Evidence/history of cancer, metabolic disease (e.g. thyroid disease, diabetes) or cardiovascular disease (e.g. coronary artery disease, myocardial infarction, stroke, atherosclerosis), or disease that may influence metabolism
- Current use of prescription medication or other drug that may influence metabolism (diet/weight-loss medication, asthma medication, psychiatric medications such as anti-depressants, anti-anxiety medications, and stimulants for ADHD, corticosteroids or other medications at the discretion of the PI and/or study team)
- Pregnancy, lactation at any time during study/follow-up period (women only)
- Evidence of vigorous exercising in order to lose weight, change body shape, or to counteract the effects of eating
- Previous bariatric surgery
- Evidence of nicotine dependence as determined by Fagerstrom score ≥ 3 (including chewing or smoking tobacco), any drug use (amphetamines, cocaine, heroin, marijuana), or problematic alcohol use (i.e. diagnosis of alcohol use disorder: meeting ≥ 2 of 11 criteria in past 12 months, ranging from drinking more/longer than intended to experiencing withdrawal symptoms)[13]; report of binge drinking: ≥ 5 drinks in 2 hours or ≥ 4 drinks in 2 hours for men and women, respectively) over the previous 6 months.
- Volunteers with strict dietary concerns (e.g. kosher diet, milk allergy or lactose intolerance, or food allergies)
- Caffeine consumption > 300 mg/day (roughly $\geq$3 cups coffee or 2-3 energy drinks)
- Having metal implants incompatible with MRI (for example, pacemakers, metallic prostheses such as cochlear implants or heart valves, shrapnel fragments, etc.).
- Having had previous radiation exposure within the last year for either medical or research purposes (e.g. X-rays, PET scans, etc.) that would exceed research limits. Excessive radiation exposure will be determined at the discretion of the PI and/or study team
- Are claustrophobic to a degree that they would feel uncomfortable in the MRI machine.
- Non-English speakers.
- Cannot commit to the schedule of visits to the Clinical Research Center as required by the study timeline

2.3 Study Schedule

2.3.1 SCREENING PROCEDURE (PHASE A)

Several screening procedures will be conducted remotely when the volunteer is at home. First, subjects will complete a telephone screening with a member of the research team to assess eligibility for the scanning portions of the study. Interested individuals will be provided an overview of the study during the telephone screening, and they will be asked to verbally consent to completing the screener questions. If the interested individual is either ineligible for the study or does not wish to participate, their responses will be destroyed.

Volunteers who have been deemed eligible based on the telephone screen and who wish to proceed with the study will be sent an advance copy of the consent form and will be invited to the NIH Clinical Center to provide written informed consent and complete a screening visit consisting of a medical history evaluation, a physical examination, and brief cognitive testing. To the extent possible, portions of the screening visit may be completed via telehealth appointment.

Screening sessions will begin in the morning, and subjects will be required to complete an overnight fast before the screening tests. Upon arrival at the NIH Clinical Center, volunteers will provide informed consent. All subjects who have provided consent for participation will have the following procedures and tests performed, detailed below:

- Medical history* (including food allergies or intolerances) and limited physical examination
- Fasting blood test
- Urinary drug test
- Body weight, height and body mass index (BMI) determination
- Food frequency questionnaire*
- DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure*
- Eating Disorder Examination Questionnaire*
- MRI Safety Screener*
- Cognitive task (National Adult Reading Test - Revised (NART-R)*
- Questionnaire (Fagerstrom Nicotine Dependence Test (FNDT))*

For those participants who provide telephone consent, telehealth visits will be subsequently conducted and the above procedures that are indicated with asterisks will be collected remotely. The remaining screening procedures will be conducted in person.

A non-research, fasting blood sample will be then obtained via venipuncture, and a routine blood test will be performed on the sample to measure metabolic functioning (e.g., blood lipid profile, liver panel, electrolytes, thyroid function, blood count) to determine eligibility based on items in section *2.2 Inclusion and Exclusion Criteria*. A copy of the results of this bloodwork will be reviewed with and provided to participants to discuss with their personal physician. Additionally, participants will be asked to provide a urine sample to screen for recent use of amphetamines, cocaine, heroin, and marijuana. The results of these tests will be used to ensure study eligibility.

Body weight and height will be measured. Volunteers will meet with a clinical research dietitian to complete a food frequency questionnaire to obtain a measure of the nutritional content of the participants diet over the past year. The dietitian will also review available menu selections to

facilitate designing the diet for dietary stabilization and inpatient portions. Lunch will be provided following the morning assessments.

Participants will complete the DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure [22] and Eating Disorder Examination Questionnaire (EDE-Q) [14]. Any clinically significant medical findings will be communicated to the subject by a qualified member of the research team and appropriate follow-up with their primary care physician will be planned.

Subjects will also be asked to complete the Fagerstrom Test for Nicotine Dependence (FTND) [15], which will provide an estimate of nicotine dependence for interested volunteers who report tobacco use. As nicotine withdrawal has been associated with altered functional activity in the brain [16], the FTND will allow the research team to exclude such subjects.

Volunteers will also complete a reading assessment to estimate their IQ: the National Adult Reading Test (Revised) (NART-R) [17]. Scores from the NART are validated measures of cognitive performance that may be used to estimate IQ. As IQ has been associated with differences in brain structure and function [18], subjects with an estimated IQ < 70 (indicative of a pervasive developmental disorder) will be excluded from the study. If excluded on the basis of IQ estimation from the NART, participants will be informed that they did not meet study inclusion criteria on the basis of their "reading test results".

Subjects will also complete a screener form to ensure the safety of their participation in the MRI portion of the study. Any indication of implants containing ferrous metal will exclude subjects from participation in the study.

If qualified for entry into the study on the basis of (1) inclusion/exclusion criteria and (2) the study team's assessment of the subject's likelihood to adhere to study procedures, the research team will communicate with the subject to schedule subsequent study sessions.

2.3.2 DIETARY STABILIZATION (PRE-PHASE B)

To ensure consistent macronutrient consumption prior to PET scanning, subjects will be required to consume standardized meals designed for each participant by a registered dietitian for up to 5 days prior to inpatient testing visit and instructed to avoid consumption of other foods. All meals will be prepared by the NIH Clinical Center Metabolic Kitchen staff and provide sufficient energy to maintain body weight (estimated using Mifflin-St.Jeor equation with appropriate activity factor) via approximately 50% of calories from carbohydrate, 35% from fat and 15% from protein. Subjects will be provided a cooler and ice packs to transport meals, which will be picked up from the Clinical Center. To monitor dietary compliance, subjects will be asked to complete daily food logs, return any uneaten food and to report the consumption of any foods they consume outside of the meals prepared for them. Subjects will be permitted to continue usual caffeine consumption (coffee/tea/diet soda) but asked to refrain from alcohol consumption for 5 days during the stabilization period before inpatient admission(s). Degree of adherence to the outpatient diet will be considered when inviting interested participants to continue with inpatient phase of study.

At the beginning of this phase, subjects will be asked to provide a fasting blood sample so that we may measure metabolites of interest (e.g. plasma fatty acid amides). A sample will be collected prior to inpatient admission in order to minimize any potential influence of diet as dietary standardization may suppress natural variability in these metabolites related to the subject's free-living diet.

2.3.3 INPATIENT NEUROIMAGING SCHEDULE (PHASE B)

Based on the screening visit (Phase A), consenting volunteers will be scheduled for study Phase B (inpatient visit to the MCRU at the NIH Clinical Center), preceded by up to 5 days of dietary stabilization as described above. This phase (B) of the study will occur as up to 6 contiguous inpatient days (depending on facility availability). To control for the impact of gonadal hormones on food intake, every effort will be made so that female subjects of reproductive potential will be admitted during the follicular phase of their menstrual cycle.

Following admission for Phase B, volunteers will undergo metabolic measurements consisting of: body weight and height; measurement of body composition (e.g., dual energy x-ray absorptiometry (DEXA), air-displacement plethysmography (BodPod)) and they will complete a battery of self-report questionnaires and computer tasks related to mood, stress, thoughts about food and eating, and self-control. Female subjects of reproductive potential will also complete a urine pregnancy test on Day 1 of the inpatient visit and interviewed about the possibility of pregnancy. Positive test results will preclude subjects from further participation. The purpose of these measurements is to facilitate examination of exploratory aims by providing a comprehensive assessment of whole-body composition in the subjects, as well as measures of cognitive processes that subserve typical food intake and feeding behaviors. The baseline measurements will then be compared with similar measurements during the 1-year follow-up. Except where noted, subjects will be provided a standard breakfast, lunch and dinner (approximately 50% calories from carbohydrate, 35% from fat and 15% of calories from protein) along with coffee/tea or diet soda in accordance with their usual intake.

Subjects will complete an overnight fast prior to PET scanning for all inpatient visits. Each subject will complete two PET sessions under the following conditions: (1) fasted state with [^{18}F]fallypride, (2) [^{11}C]raclopride while fasted and post-milkshake challenge. The order of the PET scans using [^{18}F]fallypride and [^{11}C]raclopride sessions will be pseudo-randomized (i.e., random scan order will be requested but final schedule ultimately depends on PET department scheduling/capabilities of radiopharmacy production). Prior to each PET scan session (i.e., fasted state), a blood sample will be collected via venipuncture to enable measurement of circulating satiety hormones. Additionally, subjects will be asked to report subjective stress levels and craving for food at this time, measured using a Visual Analog Scales (VAS).

PET scanning will begin in the morning, and, as diurnal variation may affect radioligand binding potential, time of day will be consistent across PET conditions. The [^{18}F]fallypride scan session is expected to last approximately 3.5 hours, and subjects will be provided two scheduled breaks from scanning. Within the [^{11}C]raclopride session, both [^{11}C]raclopride scans will be about 60 min in length each, separated by a rest period of at least 60 minutes during which participants will be encouraged to void to empty the bladder, and consume 250 mL of milkshake. The second dose of [^{11}C]raclopride will be administered at least 60 min following the baseline dose. At three times (before, midway through (during a scheduled break) and near completion of Fallypride and Raclopride scan sessions), a nurse will draw a blood sample from subjects via catheter to assess any change in post-absorptive signals. Participants will be encouraged to drink water at the conclusion of each scan. An *ad libitum* test meal will be provided following completion of all scan days.

Participants will also undergo up to two MRI sessions during their inpatient stay. Prior to one scan, participants will undergo a blood draw to provide a research sample as well as blood for genetic testing. Each scan will be no more than 2 hours in duration and may consist of structural

and functional brain imaging as well as measurement of neurometabolic profile, depending on facility availability.

While at the MCRU, after an overnight fast, the participant may undergo indirect calorimetry to assess resting energy expenditure. The participant may be dosed with doubly labeled water and initial urinary samples may be collected. A member of the research team will instruct the participant on procedures for urinary collection, activity and weight monitoring during the outpatient phase (Phase C). The participant may be provided with accelerometers and a scale to track body weight at home. A member of the research team will discuss with the subject to schedule any remaining neuroimaging sessions, if applicable. Female participants will be scheduled to complete scanning, ideally within the follicular phase of their menstrual cycle to control for the effects of gonadal hormones on feeding behavior. Because inter-individual variability in menstrual cycle length has been reported, follicular phase will be adjusted by the average length of each woman's menstrual cycle. On average, the follicular phase in adult women is 15 days in length, so every effort will be made to admit women for their inpatient stay within 10 days of the first day of menstruation.

2.3.4 POST-ADMISSION OUTPATIENT MONITORING (PHASE C)

In order to examine dopamine D2BP in relation to free-living activity levels and energy balance, participants may be asked to collect daily urine samples as well as monitor weight and activity (via NIH-provided Wi-Fi-enabled, commercial digital scale for home use and research-grade actigraphs) for a predetermined number of contiguous days post discharge. The subjects would be provided equipment for the collection and shipment of urine samples, which will be collected at home for a predetermined number of days following the doubly labeled water dose. Actigraphs would be returned to NIH at the end of this period.

2.3.5 ONE-YEAR FOLLOW-UP (PHASE D)

In order to investigate the correlates of brain dopamine D2BP such as free-living physical activity, ad libitum meal consumption, and body weight and brain changes over a longer-term, participants may be asked to provide select data intermittently over the following 12 months and return to the clinical center for more comprehensive repeat assessment.

For the 12-month follow up period (Phase D), subjects may be asked to use the provided scale to log daily weights over 12 months. Subjects may also be provided with commercial activity monitors to track daily physical activity for 12 months. Four times over the year, for a duration of 7 days each collection period, subjects may be mailed research-grade activity monitors (actigraphy) for daily use and be instructed to return the devices by mail at the end of 7 days. Subjects may also be asked to complete and return surveys/questionnaires (concerning eating behavior, and activity) during quarterly collection periods.

At 12 months (± 2 months) following completion of the inpatient PET portion, subjects may be invited back to the NIH Clinical Center to complete one outpatient visit consisting of repeat MRI session, fasting research blood sampling, body composition measurement (e.g., DEXA, BodPod, BIS), computer tasks and self-report questionnaires. Subjects would be provided with lunch during their 1-year follow up visit.

To the extent possible, portions of the follow-up visit may be completed remotely and/or via telehealth appointment.

3. Analytical Procedures

3.1 PET imaging

3.1.1 PRE-SCAN PROCEDURES

1. *Fasting.* Subjects will be required to complete an overnight fast prior to all PET scan sessions.
2. *Review of scanning protocol.* A member of the research team will review the procedures to be completed during the scan session and answer any questions from the subject. Subjects will be reminded that they will be provided rest periods throughout the session.
3. *Blood sampling.* A venous blood sample may be collected via catheter ~30 minutes prior to the start of each PET scan for assessment of metabolite and hormone concentrations. A second blood sample may be collected during a scheduled break in the Fallypride scan as well as immediately prior to the second Raclopride injection. A final blood sample will be collected near the conclusion of both scan sessions.

3.1.2 PET DATA ACQUISITION: [18F]FALLYPRIDE AND [11C]RACLOPRIDE/MILKSHAKE-CHALLENGE

Dopamine type-2 receptor availability will be evaluated by PET scans that will be performed on a dedicated head scanner, the High-Resolution Research Tomograph (HRRT) with ~2.5 mm resolution. [^{11}C]raclopride and [^{18}F]fallypride are the radiotracers that will be used to determine dopamine D2 receptor availability. [^{18}F]fallypride is a selective, high-affinity dopamine D2 receptor antagonist, and [^{11}C]raclopride represents an alternative dopamine D2 receptor antagonist with moderate affinity. [^{18}F]fallypride is administered under the authority of an IND from the FDA #70,046; [^{11}C]raclopride is administered under IND #54,135. Both INDs are sponsored by the NIH Clinical Center, with Dr. Virginia Guptil, Director of Office of Research Support and Compliance (ORSC), as the sponsor's authorized representative. Prior to each scan, a swimming-like cap with small light reflectors will be placed on the subject's head. The cap is used to monitor the position of the subject's head during the scan. Information about head motion is used in the image reconstruction process to reduce the blurring effect of head movement on the PET images. Transmission scan information will be obtained over about 10 min with a 137Cs rotating pin source before each radiotracer injection to correct emission scans for attenuation. The radiopharmaceutical will be injected over a period of approximately 1-2 minutes using an FDA-approved infusion pump.

Upon completion of each scan, subjects will be asked to void to minimize radiation exposure to the bladder. We will control the environmental stimulation to the subjects to ensure that it is standardized for all subjects (noise kept to a minimum and room dimly lit). To estimate dopamine D2 receptor availability we will obtain the time-activity curves for both [^{11}C]raclopride and [^{18}F]fallypride in brain regions of interest, and tracer kinetics will be fit using a multi-compartment model with the cerebellum as a reference region.

Immediately following measurement of dopamine D2 receptor availability using [^{11}C]raclopride measured in a fasted state, an additional scan will be completed following the consumption of a milkshake (described in detail in the next section).

[^{18}F]fallypride PET Scan (Fasting):
Subjects will begin preparation for scanning at ~ 9:00 AM when a fasting blood sample may be collected immediately following intravenous catheter insertion. The subject will be placed in the PET scanner following potential blood sampling, and the transmission (attenuation) scan will be performed. Subjects will receive an intravenous injection of approximately 5 mCi [^{18}F] fallypride. Acquisition of dynamic PET data will begin with radiotracer injection, lasting about 3.5 hours, separated by two scheduled breaks for the participant's comfort. Blood will be drawn via catheter during one of the scheduled breaks and again near the conclusion of the Fallypride scan.

[^{11}C]raclopride PET Scan (Fasting/Baseline):

Subjects will begin preparation for scanning at ~ 9:00 AM when a fasting blood sample will be collected immediately following intravenous catheter insertion. Following blood sampling the subject will be placed in the PET scanner and the 10-minute transmission (attenuation) scan will be performed. Subjects will then be injected with approximately 20 mCi [^{11}C]raclopride. Acquisition of dynamic PET data will begin with radiotracer injection, lasting about 60 min.

Session Break + Milkshake:

Upon completing the scan, subjects will be asked to void to minimize radiation exposure to the bladder. As described above, environmental stimulation will be controlled throughout the scan and standardized for all subjects.

Subjects will be provided a rest period following the initial PET scan. During this time, all subjects will be given a standard milkshake (approximately 250 cc of standardized milkshake [420 Cal]) to consume prior to the second PET scan. Subjects will be given 10 minutes to consume the milkshake and be asked to rate the pleasantness of the milkshake using a visual analog scale.

[^{11}C]raclopride PET Scan 2 (Test Scan):

The second administration of [^{11}C]raclopride will occur approximately 30 minutes after the test meal is completed, which will be at least 60 min after completion of the first scan. PET scanning will begin with a blood sample collection via catheter, 10-minute transmission (attenuation) scan followed by the second intravenous bolus injection of approximately 20 mCi [^{11}C]raclopride, and dynamic PET data will be acquired for about 60 min. The binding potential of [^{11}C]raclopride for each scan will then be estimated by mathematical modeling, and dopamine release due to the test meal will be calculated from the percent reduction in binding potential between the two [^{11}C]raclopride scans (Wong and Brašić, 2001).

3.1.3 POST-SCAN PROCEDURE

A third blood sample will be collected around the time of scan completion. Subjects will be evaluated for safety, and after their final PET scan session, the catheters are removed. All subjects will be asked to void to minimize radiation exposure to the bladder. Subjects will be encouraged to drink water upon completion of the scan. Subjects will be provided a eucaloric standard diet for the remainder of the day.

3.2 MRI Data Acquisition

MRI studies may be performed by both the Biomedical & Metabolic Imaging Branch (NIDDK) on a 3.0T Siemens Verio whole body instrument and/or the NMR Center on a GE 3T

scanner. A 70 cm tube resonator will be used for radio frequency transmission and detection. Padding will be used to minimize motion. Including clinical images to be read by radiology, MRI portion will take less than 120 minutes. The participant will be asked to provide a venous blood sample prior to MR imaging. Imaging may take place 3-5 hours after a standard meal, depending on facility availability.

We will be using the MRI for investigational research. This means that the way the MRI is generating the images may be different than what is normally done in a routine clinical scan. However, all studies done under this protocol will be performed within FDA safety guidelines. To collect images for research, we will use standard clinical coils (antennae). These are parts of the machine that help generate the image. Additionally, we will use clinical MR machines used within the FDA safety guidelines.

3.2.1 ANATOMICAL MRI

Structural MR images will be obtained with a T1-weighted axial 3D modified driven equilibrium Fourier transform, and a T2-weighted coronal hyper-echo pulse sequences. This high-resolution scan will provide the basis for localizing courser-resolution PET data onto the participant's own anatomy. We will also obtain structural images using a magnetization transfer (MT) imaging that is based on the balanced steady-state free precession method (Gloor, Scheffler and Bieri, 2008). These images will be contrasted with T2-weighted data to determine white matter integrity.

3.2.2 FUNCTIONAL MRI

fMRI will be collected depending on availability of facilities. Facility schedule permitting, the following fMRI data will be collected using a coronal single-shot gradient-echo echo-planar imaging (EPI) sequence.

Neural bases of food stimulus perception. To evaluate the neural bases of food stimulus perception, subjects may be presented with photographs of four different types of foods: high-fat savory foods, low-fat savory foods, high-fat sweet foods, and low-fat sweet foods. Presentation of the food stimuli will be blocked by food category. Each block will contain photographs of food stimuli from a given category, each presented for 2.5 seconds with a 500 ms interstimulus interval. In addition to food picture blocks, subjects will also be shown blocks of non-food object photographs that will serve as an object perception control condition. The participants' task while viewing the stimuli will be simply to press a button on a hand-held response box anytime they see the same picture presented twice in a row. Picture presentation blocks will be separated by 10-second 'null-stimulus' blocks in which only a small fixation mark will be presented on the screen. These null periods will be included to allow the blood oxygenation-related fMRI signal to return to baseline after each stimulus block. Each run will be completed three times, for a total duration of about 18 minutes.

By comparing the activity associated with viewing food- and non-food stimuli, we will localize in each subject the brain regions supporting food perception in general. In addition, by comparing activity among the four food stimulus categories, we will localize ROIs supporting perception of each food type. Finally, by comparing activity between obese and lean individuals, this research

design will also allow us to identify group differences in the perception of food stimuli in general, as well as among the four food stimulus categories.

Relationship between dopamine D2 binding potential and activation in brain regions supporting food preference judgments. To investigate the relationship between dopaminergic tone and neural responses to food stimuli and food preference, volunteers may perform an fMRI task in which they will provide preference ratings in response to photographs of high-fat savory foods, low-fat savory foods, high-fat sweet foods, and low-fat sweet foods. Food stimuli will be presented in a fast event-related design, with individual stimuli appearing in a pseudo-random order. Each stimulus will be presented for 5-seconds during which subjects will provide a rating to the following question:

"if given the opportunity right now, how pleasant it would be to eat this food?"

by manipulating a handheld MR-compatible scroll wheel to select a location on a number line.
To allow mathematical deconvolution of the responses to stimulus presentations, each picture presentation will be followed by the appearance of a simple 'null-stimulus' fixation mark for between 2.5-7.5 seconds. In each run, 36 stimuli are presented (9 from each food category) for 5 seconds each. Each run, including instructions, stimuli and null periods, will last 4.5 minutes. Subjects will complete 4 runs of this task for a total duration of 18 minutes.

Analyses will include using subjects' ratings on the "liking" question as covariates on the imaging data to identify brain regions supporting food preference judgments for each of the four classes of food stimuli [19]. In addition to addressing this using whole-brain imaging analyses, we will also explore how activity during food-preference judgments is modulated within the functionally-defined ROIs identified in the food perception functional localizer experiments described above.

Functional connectivity among brain regions supporting gustation and perception of food stimuli. Resting-state functional connectivity analyses will be used to examine connectivity between food perception areas and related regions. Volunteers will undergo two runs of an 8-minute "resting state" fMRI scan. During this scan subjects will be asked to stare at a fixation mark located in the center of the display, and to do their best to clear their minds and not think about anything in particular. Seed voxels for the resting-state connectivity analyses will be selected using data from food perception ROIs. Total duration for this portion is 16 minutes.

3.2.3 MAGNETIC RESONANCE SPECTROSCOPY

Magnetic Resonance Spectroscopy (MRS) can quantify a variety of neurochemicals, including various neurotransmitters and their byproducts (e.g., glutamate, glutamine and GABA) and constituents of metabolic pathways (choline and creatine). MRS will be performed to assess neurometabolic profile of brain regions of interest. The MRS scan will last no more than 2 hours and poses no additional participant risk as gadolinium, a contrast agent sometimes administered to enhance visualization of the heart and blood vessels, will again not be used during MR study scans.

3.3 Metabolic Status and Endocrine Functioning

In order to explore the metabolic and endocrine correlates of brain dopamine D2BP and neural bases of food perception and preference, participants will undergo periodic blood sampling and continuous glucose monitoring during the inpatient phase. Research blood sampling will be repeated during the 1-year follow up visit to the Clinical Center. Note that blood sample collected during screening visit will be for clinical purposes only to determine eligibility for study enrollment.

3.3.1 RESTING ENERGY EXPENDITURE (METABOLIC CART)

The measurement will be performed in the morning after a 12 h fast and overnight rest with the subject in a supine position. An indirect calorimeter with the ventilated hood technique will be used for the 30 min measurement (the first 5 minutes will be excluded from analysis). The respiratory quotient will be calculated as the ratio of carbon dioxide production to oxygen consumption. Resting energy expenditure will be calculated using the principles of indirect calorimetry using the VO_2 and VCO_2 measurements [20].

3.3.2 BODY COMPOSITION MEASUREMENTS

Assessments of body composition may be performed using the following procedures, depending on availability of equipment.

Dual Energy X-Ray Absorptiometry (DEXA) scans will be performed using the General Electric Lunar iDXA (General Electric; Milwaukee, WI, USA) to determine body composition. Participants will change into a hospital gown, remove all metal-containing objects and lie on a table while the scanner, which emits low energy X-rays, passes along the body. The scan will take approximately 10 minutes. The radiation dose is less than 0.001 rem, which is below the guideline of 5 rem per year allowed for research subjects by the NIH Radiation Safety Committee.

Assessment of body density will be obtained using an air displacement plethysmography chamber (BodPod). Subjects will be weighed and then evaluated in the chamber while clothed in a tight-fitting bathing suit or underwear and acrylic bathing cap. Final body volume will be calculated based on initial body volume corrected for the subject's thoracic gas volume as measured by the breathing circuit housed in the rear of the chamber through a disposable tube.

We will use bioelectrical impedance spectroscopy (BIS) to measure the impedance of the body. BIS spectra will be obtained to estimate total body water (TBW) and extracellular water (ECW), as tissue impedance at low frequencies is controlled by the electrical properties of ECW and at high frequencies by the electrical properties of TBW. Subjects are measured by two sets of electrodes placed on the hand/wrist and foot/ankle while lying supine on a nonconductive surface. This is a non-invasive test.

3.3.3 PHYSICAL ACTIVITY MONITORING

Subjects will be provided with commercial-grade accelerometers to measure physical activity and sleep patterns *daily* during both the outpatient portion (phase C), and daily over the course of 12 months until 1-year follow up visit (phase D).

3.3.4 BODY WEIGHT MONITORING

Subjects will be provided with a wireless scale for home use and asked to monitor body weight *daily* during both the outpatient portion (phase C), and daily over the course of 12 months until their 1-year follow-up visit (phase D).

3.3.5 DOUBLY LABELED WATER

Subjects will receive a dose of doubly labeled water at the end of their inpatient visit. We will collect a baseline (predose) urine sample after which subjects will then drink from a stock solution of a mixture of 10% enriched $H_2{}^{18}O$ and 99% enriched ^2H_2O followed by 100-200 mL tap water to rinse the dose container. Each DLW dose will be obtained from a larger DLW batch containing a mixture of 2H2O (99% enrichment) and H218O (10% enrichment). The dose of DLW will be prepared individually for each subject (proposed dosage: 1 g DLW/kg of body weight). Spot urine samples will be collected immediately prior to dosing and at 1.5, 3, 4.5, and 6 hours after dosing and daily thereafter. Daily urine samples will be collected for 1-2 weeks after each dose has been administered in order to assess isotopic enrichments of urine samples as measured.by isotope ratio mass spectrometry. The average CO_2 production rate will be estimated from the differential disappearance of the two stable isotopes in the urine samples [21].

3.3.6 BLOOD SAMPLING FOR SUBSTRATE AND HORMONE CONCENTRATIONS

Blood samples will be collected into chilled test tubes containing sodium fluoride, EDTA, and aprotinin as preservatives. Those samples for which analysis of gastrointestinal peptides will take place will also contain a dipeptidyl peptidase IV inhibitor and a protease inhibitor (e.g., 4-(2-aminoethyl benzenesulfonyl fluoride) and be collected into chilled glass tubes. All samples will be kept on ice and then centrifuged (1600 *g* for 15 min at 4°C) within 30 min of collection for isolation of plasma. After centrifugation, the plasma will immediately be frozen and stored at -80°C for later analysis.

Example plasma substrate and hormone concentrations of interest are described in **Table 3**. The results of all bloodwork from the screening visit will be reviewed with and made available to the participant to provide to their personal physician as desired. Given data from in-patient procedures are acquired for research purposes and therefore have minimal clinical utility, results from in-patient tests will generally not be provided to participants. If any procedures of this protocol show clinically significant abnormalities that may impact the health and well-being of the subjects, however, they will be notified by a qualified member of the research team. Appropriate follow-up with their primary care physician will be planned. If needed, the research team will refer subjects to a health care provider.

Table 3. List of blood measurements (*all measures plasma based except where noted)

	Metabolite/hormone
Metabolites	Triglycerides/LDL/HDL/Total cholesterol Fatty acids*(erythrocyte and plasma) Glucose Chemistry Homovanillic acid

Hormones	Insulin Glucagon Leptin C-peptide Epinephrine Adiponectin GLP-1 (active form) PYY_{3-36} Acyl- and total ghrelin GIP FGF21 TSH Triiodothyronine (T3, f-T3) Thyroxine (T4, f-T4) FSH LH Testosterone (T, f-T) Estrogen Cortisol
Markers of Inflammation	CRP IL-6 TNF-alpha

3.3.7 CONTINUOUS GLUCOSE MONITORING SYSTEM

Subjects will wear an FDA-approved continuous glucose monitor (CGM) during their inpatient admission except during MRI procedures. The device will be used to record fluctuations in blood glucose, approximately every 5 minutes. The system consists of a small sensor, transmitter, and hand-held receiver (about the size of a pager). The small sensor, with a small needle attached, will be inserted subcutaneously. The transmitter, which is attached to the sensor, will send the measured glucose to the receiver. The data stored in the receiver will be uploaded to a software for analysis. The sensor is changed once every 7-14 days. The device is calibrated every 12 hours per the manufacturer's product instructions.

CGM readings will not be made available to either nursing staff or participants. Data will be stored in the receiver for later analysis. The device may be calibrated occasionally during the participant's stay, which involves a concurrent finger-stick blood glucose assessment. If during this event a medically actionable blood glucose reading is observed during either fasting or non-fasting conditions (<40 mg/dL or > 200mg/dL), nursing staff are instructed to draw a blood sample for subsequent analysis by DLM and follow standard treatment procedures. In the absence of an observed clinically significant reading, participants complaining of glycemic symptoms will be treated to alleviate symptoms.

3.4 Neuropsychological Assessments

In order to investigate the psychological (e.g., affect, stress/well-being) correlates of brain dopamine D2BP, participants will complete various questionnaires and tasks during the inpatient

phase. Assessments conducted in advance of/during screening visit (as noted) mainly used to confirm eligibility criteria.

1. **National Adult Reading Test - Revised (NART-R) [17]:** The NART-R is a commonly used measure of intelligence for native English-speaking adults. The test comprises 50 written words in American English, and all words have irregular spellings to examine the subject's vocabulary and not their ability to apply regular pronunciation rules. NART-R scores can be used as a proxy for intelligence as measured by the Weschler Adult Intelligence Scale. This test takes approximately 5 minutes. Completed during screening visit. Participants are eligible if their estimated IQ is greater than or equal to 70 points. If excluded on the basis of IQ estimation from the NART, participants will be informed that they did not meet study inclusion criteria on the basis of their "reading test results".

2. **Eating Disorder Examination Questionnaire (EDE-Q) [14]:** The EDE is a self-report questionnaire derived from the Eating Disorder Examination clinical interview (EDE), designed for the assessment of anorexia nervosa, bulimia nervosa, and binge-eating disorder, as defined by the DSM-*5*. The questionnaire is a 28-item measure with questions pertaining to frequency of behaviors, feelings, and attitudes occurring in the past 28-days. Completion of the survey yields four subscale scores: Restraint, Shape Concern, Weight Concern, and Eating Concern, as well as a Global score incorporating all four subscales. As we aim to exclude individuals with evidence of eating disorders, subjects who receive a clinically significant score in any section will be excluded from remaining portions of the study. The questionnaire will take approximately 15 minutes. Completed during screening visit.

3. *DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure [22]:* This questionnaire is delivered during the screening visit and assesses mental health domains that are important across psychiatric diagnoses. It consists of 23 questions that assess 13 psychiatric domains, including:
   Depression
   Anger
   Mania
   Anxiety
   Somatic symptoms
   Suicidal ideation
   Psychosis
   Sleep problems
   Memory
   Repetitive thoughts and behaviors
   Dissociation
   Personality functioning
   Substance use

   Each item asks about how much (or how often) the individual has been bothered by the specific symptom during the past 2 weeks. Each item on the measure is rated on a 5-point scale. Critical items, and follow-up measures are provided for each disorder. The

questionnaire will be administered in REDCap and scored in real time. If the subject scores raise concerns about one or more disorders, the results will be discussed with a medically qualified team member who will follow up with the participant for further inquiry. Participants will be notified of any clinically meaningful to provide/discuss with their primary care physician as desired. Completed during screening visit.

4. **Positive and Negative Affect Schedule - Expanded Form (PANAS-X) [23]**: The PANAS is a widely used measure comprising 60-items assessing general states of positive and negative affect using 5-point scales (1 = very slightly/not at all, 5 = extremely). The PANAS-X measures 11 specific affects: Fear, Sadness, Guilt, Hostility, Shyness, Fatigue, Surprise, Joviality, Self-Assurance, Attentiveness, and Serenity. To assess momentary affect, participants will rate how they feel "right now at the present moment". The PANAS has high internal consistency, good convergent, discriminant, and construct validity, and is one of the most commonly used mood measures [24]. Completed during inpatient visit (phase B).

5. **UPPS-P Impulsive Behavior Scale [25]:** The UPPS-P is a 59-item self-report questionnaire used to assess impulsivity based on five subscales: urgency, premeditation, perseverance, sensation seeking, and positive urgency. Each item is rated using a 4-point Likert scale, ranging from 1 to 4: 1 (Agree Strongly), 2 (Agree Some), 3 (Disagree Some), 4 (Disagree Strongly). The questionnaire takes approximately 20 minutes to complete. Completed during inpatient visit (phase B).

6. **Pittsburg Sleep Quality Index (PSQI) [61]:** The PSQI is a 19-item, self-rated questionnaire which assesses sleep quality, latency, duration, habitual sleep efficiency and related disturbances (e.g., use of sleeping medication and daytime sleepiness) over a 1-month time interval. Scoring reliably distinguishes good and poor sleepers. The questionnaire takes approximately 10 minutes to complete. Completed during inpatient visit (phase B).

7. **Depression, Anxiety, Stress Scales (DASS-21) [26]:** The DASS-21 is a set of three self-report scales designed to measure the negative emotional states of depression, anxiety and stress. Each of the three DASS21 scales contains 7 items per scale. Subjects are asked to use 4-point severity/frequency scales to rate the extent to which they have experienced each state over the past week. Scores for Depression, Anxiety and Stress are calculated by summing the scores for the relevant items. As the scales of the DASS have been shown to have high internal consistency and to yield meaningful discriminations in a variety of settings, the scales should meet the needs of both researchers and clinicians who wish to measure current state or change in state over time (e.g., in the course of treatment) on the three dimensions of depression, anxiety and stress. Completed during inpatient visit (phase B). Although the results of this test will not be returned to the participant, scores are subject to "real time" monitoring to facilitate alerting research team that additional follow-up is needed if scores exceed pre-defined thresholds (see section 5.1).

8. **Beck Depression Inventory (BDI-II) [27]:** The BDI-II is a 21-item self-report measure that has been used extensively in both community and clinical settings to assess depressive symptoms (e.g., changes in sleeping habits, appetite or feelings of hopelessness). Responses are recorded on a 4-point Likert scale, where greater total scores indicate increases in depressive symptoms. This questionnaire lasts 5 minutes. Completed during inpatient visit (phase B). Although the results of this test will not be returned to the participant, scores are subject to "real time" monitoring to facilitate alerting research team that additional follow-up is needed if scores exceed pre-defined thresholds (see section 5.1).

9. **State and Trait Anxiety Inventory (STAI) [28]:** This self-report questionnaire consists of 60 items that assess respondents' experience of transient (state) and chronic (trait) anxiety and arousal. Subjects will complete these items using a 4-point Likert scale. This measure is completed in approximately 5 – 10 minutes. Completed during inpatient visit (phase B).

10. **Satisfaction with Life Scale (SWLS) [29]:** Satisfaction with Life Scale includes 5 items that assess general (e.g., my life is going well) aspects of subjective well-being. Completed during inpatient visit (phase B).

11. **Cohen Perceived Stress Scale (PSS) [30]:** This 10-item self-report measure for adults (ages 18 and above) assesses how unpredictable, uncontrollable, and overloading respondents find their lives. Each item is rated on a 5-point scale from 0 to 4, and greater total scores indicated increased perceived stress. Completed during inpatient visit (phase B).

12. **MacArthur Socioeconomic Status (SES) Questionnaire [31]:** The MacArthur SES questionnaire is a widely used self-assessment questionnaire. It begins with subjective social status questions, followed by questions assessing educational attainment, occupational status, income and assets. Completed during inpatient visit (phase B).

13. **Three-Factor Eating Questionnaire (TFEQ) [32]:** The three-factor eating questionnaire (TFEQ) is a self-assessment questionnaire developed to measure dietary restraint, disinhibition and hunger. The questionnaire contains 36 items with a yes/no response, 14 items with a 1-4 response scale and 1 item with a 1-5 response scale. Completed during inpatient visit (phase B).

14. **Binge Eating Scale (BES) [33]:** This 16-item self-report measure was developed to assess the presence of binge-eating pathology in obese individuals. Behavioral (e.g., quantity of food, loss of control) and cognitive (e.g., guilt, shame) features of binge eating are determined, where each question has 3 – 4 possible response items. Scores range from 0 – 48, and higher scores are indicative of greater severity. Completed during inpatient visit (phase B).

15. **Yale Food Addiction Scale 2.0 (YFAS 2.0.) [34]:** The YFAS 2.0 is a self-report questionnaire designed to assess the presence and severity of addictive-like eating. Items

were adopted from diagnostic criteria for substance use disorders, and respondents are provided with a binary 'diagnosis' of food addiction, as well as severity ratings (none, mild, moderate, severe). Completed during inpatient visit (phase B).

16. **Barrett's Impulsiveness Scale (BIS) [35]:** The BIS is a measure of trait impulsivity that has been validated in both community and patient populations, particularly among those with externalizing disorders. The 30-item questionnaire can be reduced to six first-order factors: attention, motor, self-control, cognitive complexity, perseverance, and cognitive instability. Completed during inpatient visit (phase B).

17. **Monetary Choice Questionnaire (MCQ) [36]:** This 27-item self-report assessment requires respondents to indicate a preference for monetary rewards, which can either be delivered "Now" or "Later." Consistent evidence has shown that humans value rewards less as time to delivery increases, a phenomenon known as "delay discounting," and steep discounting of rewards has been associated with impulsiveness. Degree of discounting will be calculated using either the hyperbolic *k* parameter or an area-under-the-curve analysis. Completed during inpatient visit (phase B).

18. **Fagerstrom Nicotine Dependence Test (FNDT) [15]:** This is a standard self-report measurement provides an ordinal measurement of an individual's addiction to nicotine as it is related to cigarette smoking. Binary (yes/no) items are coded as 0 or 1, and multiple-choice items are score from 0 – 3, where higher total scores indicate greater nicotine dependence. Completed during screening visit.

19. **Liking Survey**: Subjects will complete a validated, 100-item liking survey comprised of foods, beverages, physical activities, sedentary activities, pleasant experiences, and unpleasant experiences [37, 38]. The survey will take about 5–10 min to complete. Subjects will be oriented to the liking scale with examples of activities that are generally considered highly likeable (winning the lottery, succeeding), neutral (doing a routine chore), and highly dislikable (getting a paper cut, running out of money). The bidirectional, horizontal scale ranged from ''strongest disliking of any kind'' (-100) to neutral (0) and ''strongest liking of any kind'' (+100) labeled with five faces (Pallister et, 2015) Each item has a word label and a picture. The items are grouped into eighteen nutritional, sensory, or activity groups: alcohol, sweet foods, fruits, vegetables, low-fat protein, high-fat protein, sweet drinks, fats, carbo- hydrates, whole grains, salty, bitter, sour, spicy/flavorful, physical activities, pleasant experiences, and unpleasant experiences [39]. Completed during inpatient visit (phase B).

20. **Edinburgh Handedness Inventory [40, 41]**: Subjects will complete a short (~1 minute) survey in order to objectively determine whether one is left or right handed. Handedness will be used to better understand results of structural/functional brain imaging analyses. Completed during inpatient visit (phase B).

21. **Apathy Motivation Index (AMI) [62]:** Subjects will complete a short questionnaire (<10 minutes) in order to assess general motivation characterized by action initiation and goal

directed behavior. The 18-item questionnaire will be administered in REDCap and will take less than 10 minutes to complete (phase B).

22. **Big 5 Personality Test [e.g. 63]** To assess participants degree of extraversion, neuroticism, agreeableness, conscientiousness and openness to experience, participants will read statements querying their perception themselves in a variety of situations and asked to indicate the strength of agreement with each statement, choosing the best response on the scale ranging from strong disagreement (1) to strong agreement (7). The questionnaire will be administered via REDCap and will take less than 30 minutes to complete (phase B).

23. **Self reported Habit Index [64]** is a 12-item scale which assesses more behavioral automaticity than cue-dependency. Participants will complete this short (<10 minute) survey on REDCap. (phase B).

24. **Creature of Habit Scale [65]** is a 27-item questionnaire collected in REDCap that reflects variations in individuals' tendencies towards responding in a habitual manner in everyday life, particularly with respect to routine and automatic (disinhibited) responses to food (phase B).

25. **Reward based eating drive scale (RED-13) [66]** questionnaire administered in REDCap to assess reward related eating. The RED-13 is a 13 item questionnaire designed to capture more variance at lower levels of reward related eating, so it may be more sensitive to subtle changes in the reward related eating construct at the milder end of this continuum (phase B).

3.5 Computer and Laboratory Tasks

In order to investigate the psychological correlates of brain dopamine D2BP, participants will complete the following survey and computer tasks over the course of their inpatient visit.

3.5.1 INTERNAL STATE VISUAL ANALOG SCALES

Hunger and satiety assessments. At various points over their inpatient admission and outpatient follow-up visit, subjects will be asked to complete a survey to identify their perception of hunger (i.e., visual analog scale [VAS]) [42]. More specifically, the VAS survey will consist of four questions: 1) "How hungry do you feel right now?" 2) "How full do you feel right now?" 3) "How much do you want to eat right now?" and 4) "How much do you think you can eat right now?" Below each question on the survey there is a horizontal 100mm line with qualifying statements such as “Not at all”/“The least I can possibly” and “Extremely”/“The most I can possibly”, anchoring the line on the extreme left and right side, respectively. In response to each question, subjects will be asked to digitally draw a vertical mark on the horizontal line to represent the magnitude of their response to the question. A value for each response is quantified by measuring the distance of their mark (in mm) relative to the left end of the line. Therefore, the values (or "scores") for each question range from 0 to 100.

Intensity assessments. In order to assess participants’ interoceptive awareness of their GI sensations, participants will also be asked to rate the intensity of their overall sensations in their

stomach in relation to four specific stomach sensations: hunger, fullness, pain, and nausea. At various points over their inpatient admission and outpatient follow-up visit, a computerized VAS will be presented on a computer tablet, and participants will use a finger to slide a bar across the scale to indicate their subjective experience. The scales are anchored from 0 (*No Sensation*) to 100 (*Extremely Intense Sensation*).

3.5.2 LIKING & WANTING

Subjects will complete the Liking and Wanting computer assessment. The liking and wanting procedure comprises two tasks designed to assess (1) explicit liking and wanting, followed immediately by (2) implicit wanting for the same target food stimuli [43, 44]. The separate task elements will be integrated to fully randomize explicit and implicit trials. Experiment generator software (E-prime v2.0) is used to integrate the single stimulus trials for the liking task with the paired stimuli trials for the wanting task. Food stimuli presented in the procedure are selected from a database of photographic stimuli and sorted according to their fat content and taste properties into one of four separate categories: high-fat savory (HFSA); low-fat savory (LFSA); high-fat sweet (HFSW); and low-fat sweet (LFSW). Each category is represented by four different foods; hence a total of 16 different food stimuli will be presented in the procedure.

The aim of the explicit task is to obtain introspective hedonic measures for the same stimuli used in the implicit wanting task. Therefore, each food stimulus is assessed independently using visual analogue scales (VAS). The explicit computer task trials consist of 16 food stimuli presented one at a time and rated according to a 100-mm VAS anchored at each end by the statements "not at all" and "extremely". Subjects will be prompted with the statements "How pleasant would it be to taste some of this food now?" and "How much do you want some of this food**?"** Responses on the software will be recorded online and mean ratings for each food category (HFSA, LFSA, etc.) are automatically computed.

Implicit wanting is measured by a behavioral "forced choice" methodology. In this task, a food stimulus from one of the four food categories is paired with one stimulus from the remaining categories to form a series of 96 trials in which the subjects will be given the standardized instruction to select the food they "most want to eat now". In addition to recording the frequency of selections made in each category (with a possible range of 0–48), which may reveal a relative preference, reaction time (in milliseconds) of each choice is also measured. B**y** covertly recording reaction time, subjects will be unaware of implicit changes in their behavior on the task, while remaining free to determine the direction of their choices.

3.5.3 DELAY DISCOUNTING

People generally prefer immediate rewards over delayed rewards, even when the delayed reward has a higher value. The degree to which delayed rewards are discounted in comparison to immediate rewards may provide an index of impulsive decision-making. It has been reported that obese individuals may have difficulty adhering to diets because the long-term rewards of weight loss are strongly discounted when compared to immediate rewards, such as palatable food [45].

We will implement delay discounting computer procedures using an image of a food that scored highly on each individual's liking scale. This food image will be presented along with a forced choice statement such as "Would you rather eat this food now or receive $10 tomorrow?" The subject must select either the "food" or "money" option. We will also measure how long it takes to make each selection. The same food image will always be used as the hypothetical subject-specific reward, but hypothetical monetary choices will vary in the amounts of: $1, $5, $10, $20,

$50, $100 and the associated delay times will vary from: now, 4 hours, tomorrow, 1 week, 2 weeks, and 1 month.

Experiment generator software (E-prime v2.0) will be used to present the paired stimuli trials and will be programmed to center the cursor between each trial to produce more consistent response times.

3.5.4 EYE TRACKER

The ISCAN® ETL-300 Eye Tracking device provides detailed measurements about what people look at when they are presented with a visual stimulus, such as a picture of food. Depending on availability of equipment and scheduling of computer tasks, we may use this commercially available device to measure eye gaze duration, eye direction, pupil dilation, and spontaneous eye blink during the various computer tasks. As spontaneous eye blink rate has been cited as a non-invasive measure of resting dopamine release, particularly in the striatum [46], we may film subjects' eye movements at rest before and after some scanning sessions. These measurements will help us better understand the psychological factors underlying the subject's choices during the various computer tasks.

The eye tracking device can be used with contact lens or glasses. The subject's eyes are viewed using a specialized miniature infrared video camera system embedded in the headgear. A second video imaging system captures the scene that the subject is viewing. A 25-foot tether will connect the head-mounted device to a computer for data storage and analysis. Resting eye blink data will be acquired at approximately 6-minute intervals. Pilot data collected using the eye-tracker have found that the device is comfortable for subjects to wear during the computer tasks and provides implicit measures in addition to the explicit measures collected during the tasks.

3.5.5 FOOD AND NEUTRAL OBJECT GO/NO GO

Subjects will complete a Go/NoGo task, a well-validated behavioral measure of impulse control. In this task, subjects will be asked to press a button in response to target foods and withhold a response for non-target images. The order of target foods will be counterbalanced. We will use palatable (high fat/high energy; (e.g. macaroni and cheese, French fries, ice cream, cookies), less palatable (low fat/low energy; e.g., grilled chicken, yogurt, pickles, salad) and non-food targets (e.g., toys displayed on round disks) that are equated in complexity and spatial frequency to the food stimuli. In each run, subjects will be presented with a stimulus for 500 milliseconds followed by a variable inter-stimulus interval (ISI) of 2 to 4 seconds. Target stimuli will appear on 75% of the trials to build up the prepotent response pattern. Non-targets will appear the other 25% of the time and will be parametrically manipulated in order to compare non-target trials following 1, 3, or 5 target trials as in Durston et al. [47], where it was shown that, as the number of preceding target trials increases, response inhibition to non-targets becomes increasingly difficult. Participants will be given a 3-minute practice to ensure they understand and can follow the directions, followed by 192 trials (12 minutes). Outcome variables are reaction time, hit rate and false alarm rate.

3.5.6 SLIPS-OF-ACTION PARADIGM

We will use a validated version of the slips-of-action paradigm to assess subjects' reliance on goal-directed ('action-outcome') versus habit ('stimulus-response') learning [48, 49]. Goal-directed behaviors are guided by the assessment and valuation of possible outcomes. Thus, if the

outcome of a certain behavior is devalued, the behavior is less likely to be performed. Habit responding occurs when an individual's actions are driven by external stimuli, which trigger and automatic behavioral response. This task assesses such learning in three stages: control discrimination training, congruent discrimination training, and an incongruent conflict task.

In the initial stage, subjects will be presented with a cue (a box) with instructions to either "Press Left" or "Press Right." If the subject responds correctly, they will receive a reward (i.e., the box will open, revealing an image that is worth points). Following 4 practice trials, the subject will begin the next set of practice trials, where they will need to learn the correct key for the reward. Subjects will respond with either "Left" or "Right" presses. Subjects are instructed to try to win as many points as possible by responding as quickly and accurately as possible. Incorrect responses and those made after 2s will result in 0 points. This type of discrimination should be soluble by both the goal-direct and habit system.

For the second stage, two open boxes containing different icons will appear on the screen for 4 practice trials. One box will be covered with a red cross, indicating that the previously correct response is no longer associated with a reward. Subjects will be told to only respond by pressing the key that is associated with a valued image. No feedback will be provided for correct or incorrect responses. During the test trials, icons will be replaced with various new icons, and subjects must learn the correct keys as they did in the practice session. This stage of the task should be controlled predominantly by a goal-directed system.

The third stage features a conflict between the previously associated cues and outcomes. For this discrimination, the cue and outcome status of stimulus pairs has been reversed. These pairings force the subject to rely on stimulus-response learning to solve the cue-outcome incongruent discrimination. This task will be completed in ~ 72 test trials, presented in 6 blocks with an 8 second inter trial interval. Subjects will view each cue for 1s with a 2 – 2.5s response window. Including practice trials, total task time will be 27 minutes.

3.5.7 Reward Prediction Error

We will use a computerized probabilistic procedural learning task, the Probabilistic Selection Task, to assess participants ability to learn from both positive and negative outcomes [50]. Dopamine plays a key role in reinforcement learning. Learning from positive outcomes is thought to be a result of phasic bursts firing of dopamine which stimulates the D1R direct ("Go") pathway and inhibits the D2R indirect pathway ("Stop"). The dip in dopaminergic firing experienced as a result of negative outcomes is thought to support learning to avoid the unrewarded choice. This task trains participants on reward contingencies and then tests their learning strategy to determine whether they are more adept at learning predominantly from positive feedback (suggestive of high phasic dopamine response) or from negative outcomes (suggestive of sufficient degree of dip in phasic and tonic dopamine response).

In the training phase of the task, participants will learn via feedback to select one of two stimuli across 3 stimulus pairs presented randomly. Among each pair of stimuli (AB, CD, EF), feedback as to the correct stimulus is given in a probabilistic manner. Among pair AB, stimulus A will be "correct" 80% of the time and thus stimulus B will be the "incorrect" choice during 80% of forced choices (but conversely, stimulus B will be the "correct" choice during 20% of the trials). The probability distinction between correct and incorrect choices is made less stark with stimuli C and E, which are the correct choices 70% and 60% of the time, respectively. As described by Frank et al. (2004), participants will learn to choose stimuli A, C and E, more often than their counterparts

B, D, and F during training. Participants will train to criterion (65% A in AB, 60% C in CD, 50% E in EF) prior to advancing to the next phase [51].

To determine whether this learning was accomplished predominantly by learning to select A, C, or E (positive feedback) or to avoid B, D, or F (negative feedback), participants are subsequently tested using novel stimuli pairs without feedback. During this phase, participants are asked to select the stimulus that is most likely to be "correct" based on what they learned from training. Based on an analysis by Cox et al. (2015), positive feedback learning will be assessed via percentage of novel combination trials where patterns A and C were selected (A>CDEF + C>EF) while negative feedback learning will be assessed via percentage of trials where B and D were avoided (B<CDEF + D<EF). Both phases of the task combined last approximately 25 minutes.

3.6 Taste preferences and food intake

In order to investigate the associations between brain dopamine D2BP, food perception and preference and food intake, participants will complete a taste paradigm focusing on fat and sweet preference/perception as well as an *ad libitum* food array during their inpatient visit. Assessments conducted in advance of/during screening visit (as noted) mainly used to confirm eligibility criteria.

3.6.1 FOOD FREQUENCY QUESTIONNAIRE (FFQ) [52]:

Food Frequency Questionnaire (FFQ) is a self-assessment questionnaire developed to identify foods and macronutrients that significantly contribute to energy intake. Subjects will be given instructions to complete a form, which will be reviewed for completeness by clinic staff and any blank answers will be resolved. The FFQ contains questions on the frequency and portion size of consumption of 100 items over a defined period of time. We plan to use the latest version of the National Cancer Institute Diet History Questionnaire. Completed during screening visit.

3.6.2 PSYCHOPHYSICAL TASTE TEST

Participants will complete a taste paradigm to determine sucrose detection threshold, as well as sucrose and fat preference levels as follows.

Above-threshold or Suprathreshold intensity Test: Suprathrehold intensity testing allows for the assessment of the intensity of a stimulus that is clearly perceptible by the assessor between concentrations of a compound, at a suprathreshold concentration range. GLMS are designed to be more valid than traditional VAS when comparing inter-individual subjective ratings. Subjects will be trained on the use of the general labelled magnitude scale (gLMS) before we measure perceived intensities. Two trials consisting of 4 ascending concentrations of each stimulus with the first "concentration" being water will be presented to the subjects. All four concentrations will be presented in random order without repeat. Subjects will rate the perceived intensity of the stimulus using the gLMS scale and we will use the mean intensities of the two trials at each concentration for each stimulus to evaluate subjects' taste intensity perception. We will **use 0.00, 0.09, 0.35, and 1.05 mol/l** sucrose solutions. The task takes approximately 30 minutes to complete.

Preference testing: Sucrose and fat preference will be assessed using a two-series paired comparison-tracking method developed at the Monell Center for Adults [53-55]. Subjects will be presented with pairs of solutions differing in sucrose concentration (3, 6, 12, 24, and 36 g per 100 mL) and pairs of puddings differing in fat concentrations (0, 3.8, 8.4, 19, and 33 percent fat by weight, achieved via dilutions of skim 0% fat and heavy cream 33% fat in commercially available

vanilla pudding powder). They will be asked to taste the samples without swallowing and point to which of the pair they liked better. Subsequently, each pair presented will be determined by the subject's preceding preference choice. The entire task is then repeated with the stimulus pairs presented in reverse order. After completion of the taste task, the geometric mean of the preferred concentrations will be determined. This serves as an estimate of the participant's most preferred level of sweet and fat, separately [56, 57]. The task takes approximately 15 minutes to complete.

To evaluate subjects' ability to detect differences in the amounts of fat and sugar, subjects will sample without swallowing the five solutions differing in sugar concentration during one session and the five puddings that differed in fat during another. Subjects will rinse three times after tasting each sample. After each sample is tasted, they will be asked to rank them from least to most sweet or from least to most creamy, respectively. Subject responses will be scored from 0 to 2, following Stewart et al. [58]. A score of 2 indicated all correct (for example, 3.1, 6.9 and 15.6% wt/wt); a score of 1 indicated one was correct and the other two were adjacent to each other (for example, 3.1, 15.6 and 6.9% wt/wt); and a score of 0 indicated that all were incorrect (for example, 6.9, 15.6 and 3.1% wt/wt).

All taste samples will be kept chilled and brought to room temperature prior to testing. Testing will be performed after at least 2 hours of fasting and last approximately 1 hour.

3.6.3 AD LIBITUM MEAL TEST

Study participants will be presented with a standardized lunch after their PET-scan days are completed in order to provide objective measure of ad libitum food intake under fasting conditions to correlate with neural, endocrine, and metabolic factors. Subjects will be provided with a standard meal representing an excess of their calculated daily energy requirements from which to select their lunch and told to "Eat as much or as little as you want". Subjects will be limited to no longer than 30 minutes to eat their meal and the amount of time to consume the meal will be recorded. Meal items will be weighed prior to and following consumption in order to obtain an accurate measure of the amount of food, energy and nutrients consumed. Any subsequent meals (not on the final day of admission) will be adjusted to compensate for the amount eaten at lunch to maintain a constant overall daily calorie intake.

3.7 Genetic Testing

Research has identified several candidate genes and single nucleotide polymorphisms (SNPs) that are related to body mass and obesity [59]. Specifically, genome-wide association (GWA) studies of the molecular underpinnings of obesity indicate that body weight is largely influenced by genes expressed within central nervous system tissue [60]. As such, we aim to build upon these findings by collecting DNA samples to search for obesity-associated genes and variations.

DNA samples will be analyzed using blood samples collected (additional 5 mL sample). A portion of the blood samples collected from each subject will be sent to a commercial laboratory for genetic analysis of genes that are related to obesity and dopamine. All other DNA samples will be stored indefinitely at the NIH for future analyses. We plan to perform whole genome or exome sequencing with the stored DNA samples. These analyses may take place years after the samples are obtained.

Secondary Genomics Finding Service:

Secondary (incidental) findings analysis will be performed by the NHGRI-NIHCC-SGFS team under its CLIA license. The exomes or genomes will be analyzed for a particular set of variants or genes that will be determined by the SGFS team. It is critical to recognize that this evaluation is deliberately designed to have a high positive predictive analytic validity (high likelihood that the variant is truly present in the participant and is pathogenic) and very high thresholds for pathogenicity (clinical validity).

If there is a secondary variant identified (current, 2014 estimated yield is that 2-3% of participants will have such a finding), the research participant will then be contacted by the SGFS team to inform them that a genetic finding that requires confirmation with a second sample. If the second sample is positive, the NHGRI consult attending and genetic counselor will review the variant, disclose the finding to the participant, provide medical and genetic counseling for that variant, and provide referrals for further evaluation and follow-up for the proband and recommendations for testing for his/her family members.

DNA samples stored at the NIH will be kept in locked freezers in a secure location. The samples will be coded to protect personally identifiable information and the code key will be retained by the research team. Data from these samples will be deposited into a government health research database after the data have undergone QC, as indicated in this protocol's Genomic Data Sharing Plan. The information in this database will be available only to qualified researchers who have received approval from the NIH Data Access committee. No personal identifiers will be entered into the public database. We will inform subjects of the many safety measures we will use to protect their privacy.

4. Statistical Analyses

We require 39 subjects (13 within each BMI stratum) to have >80% power to determine a significant quadratic relationship between D2BP and BMI at least as large as previously hypothesized in the review by Horstsman et al. [8]. Assuming that D2BP in putamen of the control subjects was 27 as determined in our previous study [4], then the quadratic curve in Figure 1 is given by: $D2BP=-0.0291*BMI^2 + 1.9128*BMI - 1.413$. To determine the sample size requirements to detect a quadratic parameter value with a magnitude at least as large as the one hypothesized by Horstmann et al., we randomly simulated one third of subjects from the low BMI stratum according to a normal distribution (mean BMI=21.75 kg/m^2; SD=3.15 kg/m^2), another one third of subjects were selected from a normal distribution with mean BMI=30 kg/m^2; SD=4.3 kg/m^2, and the final third of subjects were selected from a normal distribution with mean BMI=40 kg/m^2; SD=4.3 kg/m^2. Then we used the above quadratic equation to calculate the D2BP value for each subject plus random noise from a normal distribution (mean=0 SD=4). This simulated sample was analyzed using regression analysis and the p-value for the quadratic term was calculated. These simulations were repeated 10000 times and the percentage of p-values greater than 0.05 determined the power to detect a significant quadratic effect of magnitude at least as large as hypothesized by Horstmann.

The 39 subjects required for the quadratic analysis above will also be sufficient to provide 89% power to replicate our previous findings of a significant positive linear association ($r = 0.45$) with a slope ≥ 0.25 m^2/kg between BMI and D2BP in caudate and putamen using [18F]fallypride with a Type I error probability of 5% ($\alpha = 0.05$) assuming a BMI standard deviation of ≥ 8 kg/m^2.

This sample size will also provide >80% power to detect a correlation of $r > 0.4$ between the binding potential of two DA D2 receptor antagonists [11C]raclopride and [18F]fallypride. Our unpublished observations found within subject correlations of $r \geq 0.6$ for repeated striatal D2BP using [18F]fallypride under different diet conditions. Computational modeling of the PET studies using the known kinetic properties of [11C]raclopride and [18F]fallypride along with various assumptions about within-subject variability in endogenous DA tone suggests that D2BP measured using these antagonists should be significantly correlated with $r = 0.66 \pm 0.0006$.

Preliminary analysis indicates that to replicate our preliminary (n=13) finding in this cohort of BMI-dependent dopamine release in the ventral pallidum (r=0.586; p=0.045), a sample size of 21 subjects distributed evenly across 3 BMI strata (to detect $r>0.6$ at $p<0.05$ and > 80% power). As this pilot data was not available prior to study onset and the intention is to replicate this finding in an independent sample, we require an additional. 7 participants in each of 3 BMI strata to achieve this aim (total of 20 participants per strata, inclusive of original 13).

5. Safety Considerations

5.1 Possible Risks and Hazards

Research-related risks in this study include those associated with study procedures, namely blood drawing, indirect calorimetry, measurement of body composition by dual energy x-ray absorptiometry (DEXA), air-displacement plethysmography (BodPod), bioelectrical impedance (BIS), administration of doubly labeled water (DLW) for measurement of average total energy expenditure, Beck Depression Inventory-II questionnaire, and positron emission tomography (PET) imaging as well as magnetic resonance imaging (MRI) scanning sessions. There are no study medications.

Blood drawing. The placement of intravenous needles may cause transient pain, and may also result in bruising, bleeding, and/or clotting at the site of needle insertion. A medical provider will be available should any of these problems occur. There is a possibility that a catheter placement would be unsuccessful or need to be removed. If this should occur, another catheter would be placed. It is possible that this may occur more than once during the subject's participation in the protocol. We will draw approximately 165mL during the inpatient phase of the study, totaling to roughly 195 mL over 12 months (including maximums of 10 mL non-research blood sampling during screening visit, 10 mL fasting research blood collected during 5-day outpatient dietary stabilization phase, 165 mL during subsequent inpatient stay, and 10 mL at 12 month follow up visit). This estimate falls in the lower end of range of approved research blood draws, which is capped at 550 mL over an 8-week period.

Continuous glucose monitoring. According to the device manufacturer, there is minimal risk associated with the device. Possible side effects include but are not limited to local infection, inflammation, pain or discomfort, bleeding at the insertion site, bruising, itching. During clinical studies submitted to the FDA upon approval of this device, there were no infections at the sensor insertion site or adhesive areas and no serious adverse events or unanticipated adverse device effects. Device related adverse events were related to sensor insertion and adhesive area irritations and to pain/discomfort during the wear period. A medical provider will be available should any of these problems occur.

Indirect calorimetry. The use of the ventilation hood may cause some minimal discomfort in claustrophobic subjects.

DEXA. The amount of radiation during the DEXA scan is less than 1 mrem to the whole body. This amount is less than the radiation exposure received by anyone living in the United States in one day. The use of the DEXA scan apparatus may cause some minimal discomfort in claustrophobic subjects and may cause some minimal back pain in a small minority of the individuals.

Air-displacement plethysmography (BodPod). This procedure may cause discomfort in claustrophobic subjects.

Bioelectrical impedance spectroscopy. There are no risks involved with this measurement and subjects will not feel the electric current.

Body weight monitoring. There are no known risks with intermittent monitoring of body weight.

Doubly labeled water. The doubly labeled water procedure has no known risks.

Eating Disorders Examination Questionnaire (EDE-Q). There is no known risk associated with this questionnaire; however, there is the potential of discovering clinically relevant information requiring further follow-up. If this is the case, subjects will be referred to their primary care for follow up with an eating disorder specialist as desired.

National Adult Reading Test (NART-R). There is no known risk associated with the NART.

Positive and Negative Affect Scale-X (PANAS). There is no known risk associated with the PANAS-X.

UPPS-P Impulsive Behavior Scale (UPPS-P). There is no known risk associated with the UPPS-P.

Pittsburg Sleep Quality Index (PSQI). There is no known risk associated with the PSQI.

Edinburgh Handedness Inventory. There is no known risk associated with the Handedness Inventory.

Ad Libitum Meal Test. There is no known risk associated with the ad libitum meal test.

Food frequency questionnaire (FFQ). There is no known risk associated with the FFQ.

Three-factor eating questionnaire (TFEQ). There is no known risk associated with the TFEQ.

Monetary Choice Questionnaire (MCQ). There is no known risk associated with the MCQ.

Barrett Impulsiveness Scale (BIS). There is no known risk associated with the BIS.

Binge Eating Scale (BES). There is no known risk associated with the BES.

Yale Food Addiction Scale 2.0 (YFAS 2.0). There is no known risk associated with the YFAS 2.0.

Beck Depression Inventory (BDI-II). There are no known risks associated with the BDI; however, one item on this measure enquires about suicidal thoughts or actions and high-ranging total score can be indicative of severe depression. This questionnaire will be administered in REDCap to facilitate "real-time" score monitoring. If a participant's score exceeds predefined thresholds, or endorses current suicidal thoughts on this measure during the study (e.g., provides a response of 1 - 3 on question 9), REDCap will be programmed to send an email alert to the study team in real-time, indicating a follow-up response is required. For patients with moderate/mild findings, the clinician may follow up with the appropriate floor's social work staff:
Social Work Department: 301-496- 2381

For patients in crisis or who have signs of suicidal ideation, the staff clinician may contact the Clinical Center's Psychiatric Consultation Liaison Service (PCLS). The social work staff may also contact PCLS if further attention is needed.

Depression, Anxiety, Stress Scales (DASS-21). There is no known risk associated with the DASS-21. This questionnaire will be administered in REDCap to facilitate "real-time"score monitoring. If a participant's score exceeds predefined thresholds, the research team will be alerted that a follow-up response is required, as described above.

DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure. There is no known risk associated with this self-report measure. However, there is the potential of discovering clinically relevant information requiring further follow-up. If this is the case, a member of the research team will contact the subject, and appropriate follow up will be planned (e.g., Outpatient Social Work consult, see above).

State Trait Anxiety Inventory (STAI). There is no known risk associated with the STAI.

Cohen Perceived Stress Scale (PSS). There is no known risk associated with the PSS.

Fagerström Test for Nicotine Dependence (FTND). There is no known risk associated with the FTND.

Satisfaction with Life Scale (SWLS). There is no known risk associated with the SWLS.

MacArthur Socioeconomic Status (SES) Questionnaire. There are no known risks associated with the MacArthur SES questionnaire although some subjects may find completing the task tedious or some may find the questions probing and too personal in nature to comfortably answer. Subjects will be informed that they do not have to respond to all the questions if they have reservations about sharing such personal information.

Internal state visual analog scales. There are no known risks associated with the visual analog scale assessment of hunger, satiety/fullness, or intensity ratings of hunger, fullness, nausea, or pain.

Delay discounting computer procedure. There is no known risk associated with the assessment of delay discounting by computer.

Assessment of liking and wanting of food. There is no known risk associated with assessment of liking and wanting of food.

Apathy Motivation Index. There is no known risk associated with this assessment of motivation.

Big 5 Personality Test. There is no known risk associated with this assessment of personality traits.

Self reported Habit Index. There is no known risk associated with this assessment of habit.

Creature of Habit Scale. There is no known risk associated with this assessment of habit.

Reward based Eating Drive Scale. There is no known risk associated with this assessment of eating behavior.

Food Go/No-go computer task. There is no known risk associated with the go/no-go computer task.

Slips of Action Paradigm/Fabulous Fruit Task. There is no known risk associated with the slips-of-action paradigm, assessed by the FFT computer task.

Liking Survey. There is no known risk associated with the liking survey.

Eye tracker. There is no known risk associated with the eye tracker device.

Reward Prediction Error Task. There is no known risk associated with the RPE computer task, though participants may find the task to be tedious.

Physical activity monitors. There are no risks associated with the monitors, but subjects may find them to occasionally be inconvenient.

Positron emission tomography. This research study involves exposure to radiation from the radioactive tracers [^{18}F]fallypride and [^{11}C]raclopride, as well as the transmission scans. The total amount of radiation subjects will receive in this study is from one injection of approximately 5 mCi of [18F]fallypride and two injections of approximately 20 mCi of [^{11}C]raclopride. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study.
Using the standard way of describing radiation dose, from participating in this study, subjects will receive a total of 6.88 rem to their gallbladder wall, 3.65 rem to their urinary bladder wall, and 3.92 rem to their liver. All other organs will receive smaller amounts of radiation. Although each

organ will receive a different dose, the amount of radiation exposure study subjects will receive from these procedures is equal to a uniform whole-body exposure of 1.22 rem. The amount of radiation received in this study is within the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is an effective dose of 5 rem (or 5,000 mrem) received per year. The average person in the United States receives a radiation exposure of 0.3 rem per year from natural sources, such as the sun, outer space, and the earth's air and soil. While there is no direct evidence that the amount of exposure received from participating in this study is harmful, there is indirect evidence it may not be completely safe. There may be a very slight increase in the risk of cancer.
A pregnancy test will be performed on the first day of admission for all female participants. If subjects are pregnant or breast feeding, they may not participate in this research study. It is best to avoid radiation exposure to unborn or nursing children, since they are more sensitive to radiation than adults.

Magnetic resonance imaging. People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. Subjects will be screened for these conditions at their outpatient screening visit and will be excluded if there is any indication of ferrous metal implants. They will also be screened again before having the scan per standard procedure and if they indicate any ferrous metal implants, they will not receive an MRI scan. In addition, all magnetic objects (for example, watches, coins, jewelry, and credit cards) will be removed before entering the MRI suite.

It is not known if MRI is completely safe for a developing fetus. Therefore, all women of childbearing potential will have a pregnancy test performed within 24 hours of scanning. The scan will not be done if the pregnancy test is positive. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing. Subjects should not have a research MRI scan if they know that they have hearing loss. Everyone having a research MRI scan will be fitted with hearing protection. If it is difficult for subjects to tolerate the confinement or the noise within the scanner they will be taken out immediately if they so request. There are no known long-term risks of MRI scans.

Genetic testing. The results of genetic testing may identify a genetic mechanism relevant to obesity or other metabolic disorders. This testing may also result in the incidental finding of a genetic condition that is unrelated to obesity or metabolism. Disclosure of this information may be upsetting to some participants. The participant may seek additional testing, genetic counseling, and relevant treatment with her/his health care providers. In addition, there is the potential for other individuals outside of the study to gain access to personal information about the participants from their genetic data. This study and the NIH have extensive safeguards in place to prevent individuals from accessing this type of information. Sequencing information and basic phenotypic information (e.g., age, sex, race) will be stored on a secure database at the NIH, where access is restricted to

pre-approved researchers. Participants will be notified if there is evidence that personal information has been compromised.

Finally, information gained from genetic testing, if used outside of the study, could negatively impact an individual's ability to obtain health insurance, life insurance, or a job. Should genetic testing lead to the diagnosis of a genetic condition unrelated to obesity or metabolism, the participant may be required to disclose this information to potential insurance providers. The Genetic Information Nondiscrimination Act (GINA) is a federal law that generally criminalizes discrimination of individuals based on genetic information by companies, group health plans, and most employers. According to this law, health insurance companies or group health plans (as of May 21, 2010) cannot request an individual's genetic information or use it to make decisions about eligibility or premiums; employers cannot use genetic information in hiring, promotion, or firing decisions, or in setting terms of employment (as of Nov 21, 2009).

The study team will discuss these circumstances with the participant, should s/he inquire about them.

5.2 Risks Related to Clinical Relevance of Test Results

As bloodwork collected during screening will include common laboratory tests used in practice and have some clinical utility, participants will receive a copy of their bloodwork involving these clinically meaningful lab results from the screening visit to provide/discuss with their primary care physician as desired. The study clinician will also review these results with them. If any lab tests, clinical interview, questionnaires or any other measurements made during the screening or procedures of this protocol show clinically significant abnormalities that may impact the health and well-being of the subjects, they will be notified by a qualified member of the research team. Appropriate follow-up with their primary care physician will be planned. Otherwise, as the majority of assessments performed during this study lack clinical utility, participants will not be informed of the results of their procedures.

Please see sections on *Genetics Testing* (sections 3.7 and section 5.1) for an explanation of risks associated with the clinical relevance of primary and secondary results from genetic testing.

5.3 Safety and Event Reporting

Adverse events, non-compliance both serious or continuing, protocol deviations both major and minor, as well as unanticipated problems are defined & described by the NIH Office of Human Subjects Research Protection policy #801, and will be reported in accordance with this policy. For observational studies that use PET IND Radiopharmaceuticals, an annual monitoring of the clinical investigation will be conducted through a program managed by the NIDDK, and the PI will provide the NIH CC (ORSC) with a copy of the monitoring report.

Serious unanticipated problems and serious protocol deviations will be reported to the IRB and CD (Clinical Director) as soon as possible, but not more than 7 days after the PI first learns of the event. Regarding IND usage, an investigator must immediately report any serious adverse event within 7 working days of the PI awareness of the event to the IND sponsor. This will be done using the MedWatch Form 3500a, whether or not the event is considered drug related, and must include an assessment of whether there is a reasonable possibility that the drug caused the

event. In the event that there is evidence suggesting a causal relationship between the drug and a serious adverse event (e.g. death from anaphylaxis), the investigator must immediately report the death to the sponsor at REGSupportORSC@nih.gov.

The IND sponsor will notify the FDA via phone, fax or e-mail of any unexpected fatal or life-threatening suspected adverse reactions as soon as possible but no later than 7 calendar days of initial receipt of the information. This will be followed with a written report within 15 days using the MedWatch Form 3500a.

5.4 Data and Safety Monitoring Plan

Given the modest level of risk involved in the research procedures, no Data and Safety Monitoring Board will be instituted. Subjects will be monitored for safety by the Medically Accountable Investigator. Adverse events will be recorded and reported as specified in section 5.3 by the Principal Investigator.

The study will be subject to audits to ensure compliance with the protocol and applicable regulatory requirements consistent with the NIDDK Monitoring plan. Audit results will be reported to the Principal Investigator for further reporting as appropriate. Study documents and pertinent hospital or clinical records will be reviewed to verify that the conduct of the study is consistent with the protocol plan.

6. Investigator Responsibilities

The Principle Investigator, Dr. Kevin Hall, assumes responsibility for the study design, supervision of Associate Investigators and staff, and general management of subjects and PII.

Associate Investigators will be involved with distinct aspects of the protocol, and responsibilities have been outlined accordingly. Stephanie Chung, MBBS assisted by Michael Stagliano, CRNP, will oversee participant admission, physical health assessments. Juen Guo, PhD contributed to study design and will assist with data analysis along with Valerie Darcey, Ph.D., R.D. and post-baccalaureate IRTAs will assist with pre-screening, participant management, and data acquisition and analysis. The NIH CC ORSC will sponsor the PET imaging component, and is responsible for study design and data acquisition as it relates to PET.

7. Recruitment Strategies

Subjects will be recruited nationally through advertisements in clinicaltrials.gov and possibly flyers, newspapers, magazines, internet, mail, radio, television, or local mental health treatment centers. We will utilize the NIH Patient Recruitment and Public Liaison Office in establishing and implementing a recruitment strategy. We may also utilize other recruitment strategies, such as targeted ads on social media through recruitment agencies/platforms like BuildClinical. BuildClinical is a data-driven platform that helps academic researchers recruit participants for research studies more efficiently using social media, software, and machine learning. They have worked with IRBs, Research Governance Boards, and Ethics Committees in the US, UK, and Canada to ensure adherence to all the appropriate guidelines and procedures. The company uses IRB-approved content to develop a "digital footprint" and engage individuals who map to a study's profile. Once a patient expresses interest in the study, BuildClinical passes along

their information to the research through their Secure Socket Layer (SSL) software, which encrypts all inputted information, and keeps patient information private. BuildClinical's backend servers are stored at some of the most secure data centers in the world. The platform also complies with the Safe Harbor Privacy Principles of notice, choice, onward transfer, security, data integrity, access, and enforcement

The age range for inclusion in this protocol is 18-45 years at the time of recruitment. The rational for excluding subjects older than 45 years is multi-fold. First, aside from the relationship with BMI investigated in the current proposal, normative aging is associated with significant declines in dopamine D2 binding potential. Normative aging is associated with alterations in brain structure and function, particularly in response to inhibitory control tasks, which could affect image registration and analysis. Moreover, the combined effects of aging and obesity have been associated with reduced grey and white matter integrity, beginning in the fourth and fifth decades of life, respectively. Additionally, a number of the major metabolic outcome variables of this protocol have been shown to change significantly with aging (e.g., metabolic rate and body composition). Finally, much of the current information on our primary outcome measurements was gathered from subjects within the age range specified by this protocol; therefore, we believe that this age range is appropriate as it allows us to put our results into direct context with the current knowledge base.

Non-English speaking subjects will be excluded due to the use of multiple questionnaires in this study that have yet to be validated in other languages.

7.1 Recruitment of Children and Other Vulnerable Individuals

NIH employees will be eligible to participate in this study. We will use appropriate methods to recruit staff participants such as posting flyers where public announcements are permitted (rather than direct solicitation of subordinates whether via oral/written communication). Staff subject's data will be kept private and confidential. We will provide the NIH Information Sheet on Staff Research Participation to staff prior to obtaining consent to help them understand the possible consequences of their participation (see section 10.0 for details of consent monitoring for NIH staff members). Neither participation nor refusal to participate as a research subject will have an effect, either beneficial or adverse, on the staff participant's employment or position at NIH. Additional safeguards may be employed, including independent consent monitoring, training study staff on how to obtain/manage potentially sensitive information about a co-worker.

We will actively encourage the participation of women and minorities. Considering the prevalence of obesity and binge-eating behaviors in the general population, we expect women to represent approximately half of the study population. Moreover, given the increased incidence and morbidity of obesity among minorities and the social demographics of the greater Washington, DC area, we anticipate that African-Americans and Hispanics will constitute at least 20% of enrollees.

On the other hand, we have deliberately chosen not to study children/adolescents, pregnant/lactating women as well as human fetuses/neonates at this time, pending the outcome of the findings of this protocol. At present, there is no data to suggest that the binding potential of the dopamine D2 receptor antagonists, [^{18}F]fallypride and [^{11}C]raclopride, differs significantly between children, adolescents, and adults. Evidence further suggests that the effect of satiety on activity in reward regions of the brain remains stable across childhood and adulthood. As such, we see no compelling reason to expose children to the rigors and inconvenience of this type of study. If subjects are pregnant or breast feeding, they may not participate in this research study. It is best

to avoid radiation exposure to unborn or nursing children, since they are more sensitive to radiation than adults. Furthermore, only adults who are able to provide informed consent will be recruited for this study as it involves a number of cognitive tasks which require cognitive capacity.

We fully recognize, however, that the insights derived from the data analysis in this study may very well direct our attention to other potentially fruitful areas of clinical research involving childhood/adolescent or gestational obesity, given the early onset and alarming risks associated with obesity and other major public health issues in this population.

8. Subject Withdrawal

The subject may choose not to be in the study, or, if they agree to be in the study, they may withdraw from the study at any time. If a subject withdraws from the study, no new data will be collected for study purposes unless the data concern an adverse event related to the study. If such an adverse event occurs, we may need to review the subjects' entire medical record. All data, including blood or other samples, which have already been collected for study purposes, and any new information about an adverse event related to the study, will be sent to the study sponsor. A subject's decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits to which s/he is entitled, and will not affect access to health care at the National Institute of Health.

Also, there are several conditions that require the Principal/Associate Investigator to drop a study volunteer from this protocol, but not limited to the following:

- Development of any new medical condition or start of medications that would have prevented enrollment in this study as it pertains to the exclusion criteria
- Inability or unwillingness to comply with study requirements
- The subject becomes pregnant during the course of the study
- The Principal/Associate Investigator deems it unsafe to remain in the study
- The study is terminated

9. Research Use, Storage, and Disposition of Human Subjects' Samples and Data

As with all clinical data, the findings from this study will be kept confidential to protect Personally Identifiable Information (PII). Volunteer clinical data will be protected and tracked using standard operating procedures in the medical record department. All research charts and records will be kept in a secure place in a locked file cabinet in the office of the Principal Investigator. All research samples and data will be identified by a study code linked to the subject's name and the code and the results of all analyses will be kept strictly confidential. All research samples (e.g., blood, fluids) will be coded for storage in refrigerators and freezers in a locked laboratory. The plan is to store the samples until they are analyzed, and a sample of serum will be safeguarded for future analysis of other factors related to obesity and its phenotypes. These samples will be stored indefinitely. The IRB will be notified in the event these samples are accidentally destroyed, lost or are anonymized. Studies on these samples will always be related to the scope of this proposal.

Some clinically relevant research data will be stored indefinitely in the subject's medical record. This data will be accessible to the subject for review by others of the participant's choosing

(doctors, insurance companies etc.) after completing a release of information. This and other data will be maintained in databases that are password protected and secure.

9.1 Collaborations on Stored Samples

All samples and data will be transferred and coded per NIH guidelines. Any future collaborations requiring transfer of stored biological samples and data will be done after informing the IRB and obtaining necessary assurances from the outside institution. The protocol will be amended, IRB approval obtained and appropriate technology transfer agreements executed when such collaborations are established.

10. Informed Consent

Written informed consent will be obtained from the participant prior to any screening visits, study procedures or treatments. The Principal Investigator or other designated qualified protocol investigators (as indicated on the key study personnel (KSP) page) will explain the study in language understandable to the subject.". Sufficient time and opportunity will be given for discussion of the research as well as to answer any questions they may have, taking care to minimize or eliminate the perception of coercion or undue influence. The participant and the investigator will sign the current IRB-approved informed consent document.

For NIH staff members, if the individual obtaining consent is their supervisor or a non-supervisory co-worker, independent monitoring of the consent process via the CC Department of Bioethics Consultation Service or a Clinical Research Advocate from the NIMH Human Subjects Protections Unit will be used to reduce the risk of undue pressure.

A copy of the consent will be given to the subject for future reference. The signed documents will be sent to the Medical Records Department for placement in the subject's permanent CC medical record. The consent process will additionally be documented in the electronic medical record (CRIS).

Non-English speaking subjects are excluded from this study, therefore the use of the short form consent will not be required.

In most cases, consent will be obtained during a visit to NIH. However, in some cases, consent will be obtained by telephone. Telephone consent will be used under the following circumstances:

1. Patients who are unable to travel to the NIH but who wish to have their medical records or samples (including genetic samples) included in the study.
2. Patients who are already enrolled in the study, in whom updated consents/assents are needed.
3. Patients enrolled in other NIH studies who have not previously consented to this study, who do not wish to come to NIH for the sole purpose of enrolling in this study.

If a telephone consent is performed, the telephone written consent form will be mailed to the subject with a prepaid return envelope in advance of the telephone consent. The study team will arrange a time with the subject to conduct the telephone consent process. At this prearranged time, the PI or associate investigator (as listed on this protocol) will call the patient to review the details of the study to obtain the patient's verbal and written consent to participate. The subject

will sign the consent form and mail it to NIH, where it will be signed by the consenting investigator and filed in the patient's medical record and a copy will be mailed to the patient. A note will also be filed in the patient's electronic record documenting the call/consenting process. If the patient subsequently comes to NIH, the consent/assent process will be repeated in person at the next in-person visit.

In most cases, telephone consent for this study will be used for participants who are already enrolled in this or other NIH studies. If a telephone consent is performed on a subject who is not already a participant in an NIH study, the study Investigator will obtain consent to External Location Registration for the NIH Clinical Center (NIH-1225-4) from the subject. Once all consents are obtained, the study Investigator will enter the patient and study information within the Admissions, Travel Voucher (ATV) website to generate an NIH medical record number (MRN).

11. Risk/Benefits to Study Participants

This study will enroll obese and non-obese individuals. The research-related risks from participating in this study have been detailed above under "Safety Considerations" and include those associated with study procedures. While there are no direct benefits to study participation, volunteers may benefit indirectly via receipt/review of clinically useful test results (e.g., physical examination and bloodwork at screening visit) which may provide useful information about their health. Abnormal values will be discussed with the study volunteers and participants will be encouraged to continue the discussion with their primary care physicians. Clinical studies have shown that increased awareness of personal health can improve health in overweight individuals. Participation in genetic testing may not result in direct benefits to the subject. There will be no other direct benefits from participation in this study aside from the knowledge that they are contributing to advancing our understanding of the brain reward system in obesity, and that these insights may lead to new options for long-term weight management in the future.

12. Remuneration

Subjects will receive payment for the time and effort connected with the study procedures according to the table below. Note that participants may opt to complete diet stabilization portion as up to 5 days on outpatient basis (e.g. $100) or as up to 5 days while inpatient (e.g., $200) in advance of inpatient testing week.

Neuroimaging of Dopamine Transmission in Obesity

EVENT	AMOUNT	OCCURANCE	TOTAL
Outpatient per diem (1 screening visit + Up to 5 days stabilization diet)	$20	Up to 6	Up to$120
Outpatient per diem (after 1st hour)	$10	5-7	$ 50-70
Inpatient per diem (up to 5 days diet stabilization + up to 6 days testing)	$40	Up to 11	Up to $ 440
Body composition (e.g.,DEXA, Bod-Pod, BIS)	$50	1	$50
Indirect calorimetry	$25	1	$25

Blood draws (1 screening; 7 inpatient)	$20	8	$160
Continuous glucose monitoring	$20	5	$100
PET	$100	3	$300
MRI Session(s)	$100	Up to 2	Up to $200
Psychophysical Taste Test	$20	1	$20
Computer tasks	$10	5	$50
Eye movement	$10	2	$20
Psychological/cognitive testing and questionnaires	$10	19	$190
Doubly labeled water administration	$25	1	$25
Outpatient urine sampling	$10	13	$130
Physical activity/weight monitoring (outpatient)	$250	1	$250
		Total Compensation:	$1530-2150

1-Year Follow-Up

EVENT	AMOUNT	OCCURANCE	TOTAL
Outpatient per diem	$20	1	$20
Outpatient per diem (time after 1st hour)	$10	7	$70
Physical activity/weight monitoring (over 12 months)	$500	1	$500
Blood draw	$20	1	$20
Body composition (DEXA, Bod-Pod, BIS)	$50	1	$50
Indirect calorimetry	$25	1	$25
MRI Session	$100	1	$100
Computer tasks	$10	5	$50
Psychological/cognitive testing/questionnaires	$10	12	$120
Completion Bonus	$200	1	$200
		Total Compensation:	$1155

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