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

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**TITLE:** Effect of Ultra Processed versus Unprocessed Diets on Energy Intake

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## Précis

Overconsumption of foods that result from extensive industrial processing is believed to contribute to the development of obesity. Ultra-processed foods now represent most of the calories consumed in America and their contribution to overall diet has increased in parallel with the rise in the prevalence of obesity over the past several decades. While such correlations are suggestive, the effect of industrial processing per se, independent of dietary macronutrient composition, on *ad libitum* energy intake has not been carefully investigated. Therefore, we will conduct feeding studies in adult men and women to investigate the differences in *ad libitum* energy intake resulting from consuming test diets for a pair of 2-week periods in a randomized, crossover design during a single 4-week period. The test diets presented to participants will be matched for calories, macronutrient composition, sugar, fiber, and sodium, but one diet will be composed of ultra-processed foods whereas the other diet will be unprocessed foods.

## 1. Introduction and Background

The rise in prevalence of obesity and type 2 diabetes over the past several decades has been mirrored by an increasingly industrialized food system [1] involving substantial refinement of inexpensive "inputs" (primarily corn, soy, and wheat) to generate a seemingly diverse supply of convenient, stable, inexpensive, "added value" foods [2, 3] with supernormal appetitive properties [4, 5]. Ultra-processed foods [6] have becoming increasingly common worldwide [7] and now contribute to the majority of calories consumed in America [8].

Overconsumption of ultra-processed foods has been implicated as a causative factor in weight gain [9] and recommendations to reduce intake of processed foods are shared by a variety of proposed healthy dietary approaches that are widely divergent from a nutrient perspective [10]. Whereas previous studies have examined the effect of single meals varying in their degree of industrial processing on satiety and glycemia [11], no study has yet examined the effect of industrial processing on long-term *ad libitum* energy intake.

## 2. Study Objectives

In this study, we will investigate *ad libitum* food intake of 20 healthy adult volunteers who will each complete a 4-week stay at the NIH. During their stay, they will consume an ultra-processed and an unprocessed diet for 2 weeks each, in random order. We will collect information (primary and exploratory endpoints detailed below) about energy intake and metabolic changes, in response to the two test diets. These test diets that will be presented to subjects in amounts exceeding their daily energy requirements; subjects will be instructed to eat as much or as little of each diet as desired.

The diets will be designed using a standard computerized nutrition database, and foods will be selected using a food classification system that categorizes foods as belonging to one of three groups: unprocessed or minimally processed foods, processed culinary or food industry ingredients, or ultra-processed food products [6]. Diets will maximize differences in industrial processing while matching for calories, macronutrient composition, sugar, fiber, and sodium. Each subject will be admitted as an inpatient to the NIH Clinical Center and will stay on the 5SWN Metabolic Care Research Unit (MCRU).

Previous studies have successfully detected significant effects of modifying dietary fat versus carbohydrate on *ad libitum* energy intake over periods of 1-2 weeks [12, 13], We hypothesize that the degree of industrial processing will also significantly impact *ad libitum* energy intake even if the presented diets are matched for calories, macronutrients, sugar, sodium, and fiber.

Hypothesis: The hypothesis of this study is that an ultra-processed diet will lead to greater mean *ad libitum* energy intake as compared to an unprocessed diet when the meals presented to the subjects are matched for daily calories, macronutrient composition, sugar, fiber, and sodium.

**2.1 Primary Aim:**

1. To determine differences in *ad libitum* energy intake (kcals) during 2 weeks of eating an ultra-processed diet as compared to 2 weeks of an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.

Previous studies in similar numbers of subjects have shown changes in insulin sensitivity after 2-4 weeks of consuming diets that differ in advanced glycation end products that are produced through high heat cooking typical of industrially ultra-processed foods [14, 15]. One study suggested that consumption of meals composed of processed foods results in lower energy expenditure compared to consuming unprocessed foods [16]. Hepatic triglyceride content can also be altered with isocaloric diet changes in as little as 9 days [17]. Therefore, we will explore changes in glucose tolerance, various inflammatory markers, energy expenditure, and body composition metrics as follows:

**2.2 Exploratory Aims:**

1. To determine changes in oral glucose tolerance after 2 weeks of consuming an ultra-processed diet as compared to 2 weeks of consuming an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.
2. To determine differences in immune system function and inflammatory markers (c-reactive protein, E-selectin, soluble intercellular adhesion molecule-1, soluble vascular adhesion molecule-1, serum amyloid A, tumor necrosis factor-α, and interleukins 6 and 8) after 2 weeks of consuming an ultra-processed diet as compared to 2 weeks of consuming an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.
3. To determine changes in body weight and body composition after 2 weeks of consuming an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.
4. To determine changes in energy expenditure and substrate metabolism after 2 weeks of consuming an ultra-processed diet as compared to 2 weeks of consuming an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.
5. To determine changes in hepatic triglyceride content after 2 weeks of consuming an ultra-processed diet as compared to 2 weeks of consuming an unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.

**2.3 Second Cohort** The first cohort of 20 subjects completed this study in late 2018 and the ultra-processed diet significantly increased energy intake by 508 kcal/d ($p=0.0001$) compared

to the unprocessed diet – an effect that was not significantly related to sex or BMI. The increased energy intake was due to excess consumption of dietary carbohydrate and fat, but protein intake was stable between the diets.

The hypothesized mechanisms for the effect of the ultra-processed diet on energy intake include:

i) The non-beverage energy density of the meals (calories per unit mass) was higher during the ultra-processed versus the unprocessed diet (1.96 kcal/g versus 1.06 kcal/g, respectfully). Therefore, more calories were consumed for a similar mass of food eaten;

ii) The ultra-processed meals were consumed more rapidly which may have delayed satiety; and

iii) The protein content of the ultra-processed diet was very slightly lower than the unprocessed diet (14% versus 15.6% of total calories, respectively) which may have led to increased overall energy intake to match absolute protein intake according to the protein leverage hypothesis.

We propose enrolling a second cohort of 20 subjects who will be exposed to a reformulated ultra-processed diet that more closely matches the unprocessed diet regarding non-beverage energy density and protein content. The ultra-processed diet will continue to be matched in terms of daily calories, macronutrient composition, sugar, fiber, and sodium. The ultra-processed diet will be modified to include foods that are typically eaten more slowly. If these factors were the main reasons that original ultra-processed diet led to the observed ~500 kcal/d increase in energy intake over the unprocessed diet, then the reformulated ultra-processed diet should lead to a smaller difference in energy intake between diets. Except for dietary formulation, the study design, procedures, and primary and exploratory aims, will be identical in the 2 cohorts. We will also compare the differences in ad libitum energy intake between ultra-processed and unprocessed diets between cohorts 1 and 2.

## 3. Study Design

This is a randomized order crossover study of healthy adult volunteers to determine differences in *ad libitum* energy intake when consuming an ultra-processed vs. unprocessed diet matched for presented calories, macronutrient composition, sugar, fiber, and sodium.

### 3.1 Subjects

Adult subjects (18-50 years of age) will be recruited to the study via the NIH Patient Recruitment and Public Liaison Office. Telephone pre-screening will exclude subjects with diabetes or any other metabolic disorders, and those who require assistance to complete activities of daily living. All others will be contacted by the protocol team to review exclusion criteria. All subjects will be fully informed of the aims, nature, risks, and potential benefits of the study prior to giving written consent.

### 3.2 Inclusion and Exclusion Criteria

*Inclusion criteria:*

1. Adults age 18-50 years, male and female
2. Weight stable (< ± 5 % over past 6 months)

3. Body mass index (BMI) $\geq$ 18 kg/m$^2$
4. Willing to cease their habitual caffeine intake during the study, beginning one week prior to inpatient admission
5. Written informed consent
6. Willing to eat the food provided in the study
7. Able to complete daily bouts of stationary cycling at a moderate rate and intensity with a HR equal to or greater than $0.3\times(220\text{-age-}HR_{rest})+HR_{rest}$ but not exceeding $0.4\times(220\text{-age-}HR_{rest})+HR_{rest}$ and no signs of arrhythmia

*Exclusion criteria:*

1. Evidence of metabolic or cardiovascular disease, or disease that may influence metabolism (e.g. cancer, diabetes, thyroid disease)
2. Taking any prescription medication or other drug that may influence metabolism (e.g. diet/weight-loss medication, asthma medication, blood pressure medication, psychiatric medications, corticosteroids, or other medications at the discretion of the PI and/or study team)
3. Hematocrit < 34% (women only)
4. Hematocrit < 40% (men only)
5. Pregnancy, lactation (women only)
6. Participating in a regular exercise program (> 2h/week of vigorous activity)
7. Caffeine consumption > 300 mg/day
8. Regular use of alcohol (> 2 drinks per day), tobacco (smoking or chewing) amphetamines, cocaine, heroin, or marijuana over past 6 months
9. Eating disorders and psychological conditions such as (but not limited to) claustrophobia, clinical depression, bipolar disorders, that would be incompatible with safe and successful participation in this study, as determined by investigators.
    a. Past or present history of claustrophobia since part of the protocol will involve being confined to a small room for whole-body indirect calorimetry and being in an MRI scanner for liver fat measures
10. Implants, devices, or foreign objects implanted in the body that interfere with the Magnetic Resonance procedures
11. Volunteers with strict dietary concerns (e.g. vegetarian or kosher diet, food allergies)
12. Volunteers unwilling or unable to give informed consent
13. Non-English speakers due to unavailability of required questionnaires in other languages

**3.3 Study Schedule**

3.3.1 Screening Procedures

The Principal Investigator or Associate Investigator will discuss the nature of the study and answer any questions the subject may have. All subjects will be fully informed of the aims, nature, risks, and potential benefits of the study prior to giving written informed consent.

Potentially eligible volunteers will be invited to the NIH Clinical Center for a screening visit that will include a medical history and physical examination and other screening procedures. Subjects will be required to fast for at least 12 hours before the first screening visit, which will include a blood draw for assessment of blood lipid profile, liver panel, electrolytes, and blood count. The blood draw will also be used to screen for the presence of diabetes, which will be defined as a fasting glucose > 126 mg/dl or %HbA1c > 6.5%. Female subjects of reproductive

potential will complete a urine pregnancy test during this visit, and positive test results will preclude subjects from further participation.

All subjects who are interested in participating in the full study protocol will have the following procedures and tests performed:

- Medical history (including food allergies or intolerances) and physical examination
- Resting electrocardiogram (EKG)
- Resting energy expenditure (REE)
- Fasting blood tests
- Body weight and height
- Submaximal Exercise EKG
- One 20-minute stationary bicycling session with predetermined speed and resistance identified using the results from the resting and exercise EKG tests.
- Questionnaires
    - Food Frequency Questionnaires
    - MacArthur Socio-Economic Status Questionnaire
    - Three-Factor Eating Questionnaire
    - Profile of Mood States Questionnaire
    - Sleep Apnea Clinical Score (SACS)
    - STOP-Bang Questionnaire
    - DSM-*5* Self-Rated Level 1 Cross-Cutting Symptom Measure [18]
    - Owl and Lark Sleep Questionnaire (may also be completed during inpatient admission)

The goal of the submaximal exercise EKG is to achieve and maintain a target heart rate (HR) equal to or greater than $0.3\times(220\text{-age-}HR_{rest})+HR_{rest}$ but not exceeding $0.4\times(220\text{-age-}HR_{rest})+HR_{rest}$ and show no signs of arrhythmia. The 20-minute stationary cycling bout in the prescribed range will be used to assess ability to perform a minimal amount of exercise and also to determine and practice the speed and intensity for the inpatient admission. This speed and intensity will be repeated on days of scheduled cycling exercise during the inpatient visit. Any clinically significant findings from the screening procedures 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. The food frequency questionnaires (FFQ) will be analyzed to help identify any dietary restrictions or food avoidances as well as to ensure that the subject's habitual diet meets the inclusion/exclusion criteria.

Volunteers will be asked to sample the diets during this visit. They will eat foods from the Ultra Processed and Unprocessed menus supervised on the metabolic unit. If qualified for entry into the study based on the inclusion/exclusion criteria and the study team's assessment of their likelihood of adherence to study procedures, study staff will arrange for active participation in the study as soon as the next day.

3.3.2 Study Timeline

Up to 20 healthy volunteers will be studied as inpatients to the MCRU. The outline of the study is shown in Figure 1. Every effort will be made to adhere to the proposed timelines, but some flexibility is required for scheduling of other studies, unanticipated equipment

maintenance, etc. Scheduling variations will not be reported. For a calendar overview of the proposed timeline, please see Appendix A.

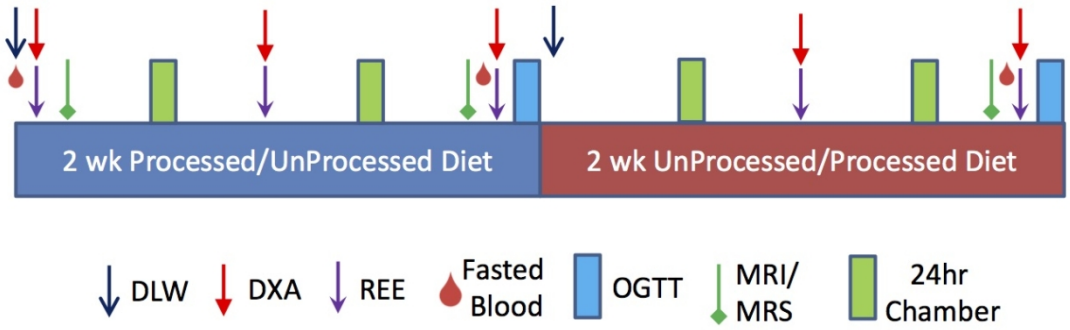


**Figure 1. Study outline.**

Subjects will complete the following procedures during the study period:

- Fasting blood draw
- Doubly labeled water dosing
- Resting energy expenditure (measured by indirect calorimetry using a bedside metabolic cart)
- Body fat by dual energy X-ray absorptiometry (DXA; whole body)
- Metabolic weight and height
- Liver fat content by magnetic resonance spectroscopy
- 24-hour respiratory chamber
- Oral glucose tolerance test (OGTT)

All subjects will have daily body weight measurements and will wear accelerometers throughout the study except during bathing. Subjects will also be required to perform exercise on a stationary bicycle at a constant pace and intensity (as determined at the screening visit) for a total of 60 minutes (recommended to occur in three 20 minute blocks) every day during their inpatient stay. Subjects will spend one 24-hour period each week residing in a respiratory chamber and will have liver fat content measured towards the end of each diet period. A baseline DXA scan will be obtained, followed by weekly DXA scans for a total of 5 scans. Similarly, the indirect calorimetry measurements will be obtained at baseline and on a weekly basis. An oral glucose tolerance test (OGTT) will be performed at the end of each diet period. During the study period, female subjects of reproductive potential will also complete weekly urine pregnancy tests prior to DXA scans, and positive test results will preclude subjects from further participation.

Participants will consume 3 meals per day in the metabolic unit. Meals will be *ad libitum* and consumed in the metabolic unit. After meal times, all meal trays will be checked, and uneaten food will be weighed. Dietitians and research staff will meet with the subjects regularly to discuss the diet and assess compliance. Subjects will be provided with snacks corresponding to the prevailing diet assignment and will keep a record of all foods consumed outside the meal times.

Subjects will receive one of two test diets with 7-day rotating menus: an ultra-processed foods diet or an unprocessed foods diet. The meals presented to subjects will be matched for calories and macronutrient content. The daily macronutrient composition of both test diets will be approximately 47% carbohydrate, 36% fat and 17% protein. See Table 1 for an example of a daily

menu for each diet. All subjects assigned to the same diet will receive identical meals (e.g., see Table 1) with instructions to eat as little or as much food as desired. Meals will be delivered 3 times per day and subjects will be given 60 minutes to finish their meal. Snacks will also be provided every morning so that subjects have access to snacks as desired throughout the day. The food provided to the subjects will substantially exceed their estimated energy requirements (approximately 200% of energy requirements as determined by 1.6×REE measured at screening) and will likely be more than the subjects can eat. Participants will also be provided with bottled water to consume throughout the day and the amount of water consumed will be recorded.

**Table 1.** Examples of unprocessed and ultra-processed diets

| | **Unprocessed Diet** | **Ultra Processed Diet** |
|---|---|---|
| Breakfast | Greek yogurt parfait with strawberries, blueberries and granola with walnuts<br>Banana | Pancakes with margarine and syrup<br>Turkey sausage<br>Tater tots<br>Apple juice |
| Lunch | Spinach salad with chicken, apples, bulgur, sunflower seeds and grapes<br>Vinaigrette | Turkey sandwich with American cheese and mayo on white bread<br>Baked potato chips<br>Diet ginger ale |
| Dinner | Beef tender roast<br>Rice pilaf (basmati rice with garlic, onions and peppers)<br>Steamed broccoli<br>Side salad with balsamic vinaigrette<br>Oranges<br>Apple slices<br>Pecans | Cheeseburger<br>French fries and ketchup<br>Diet ginger ale<br>Greek yogurt<br>Canned peaches in heavy syrup |

## 4. Analytical Procedures

### 4.1 Resting energy expenditure

Resting energy expenditure (REE) will be measured in the morning with the subject in a supine position. Subjects will be instructed to complete a 12-hour overnight fast prior to each scheduled REE. An indirect calorimeter with the ventilated hood technique will be used for the approximately 30-40 min measurement, where the first 5-15 minutes will be excluded from analysis. The respiratory quotient will be calculated as the ratio of carbon dioxide production to oxygen consumption. Resting metabolic rate will be calculated using the principles of indirect calorimetry using the $VO_2$ and $VCO_2$ measurements [19].

### 4.2 Respiratory chamber

Subjects will be in the chamber for a total of 2 days during each diet treatment period. The respiratory chamber is a specially constructed room to assess the metabolism of subjects for a period of 24 hours. Designed as a walk-in “pull” calorimeter, it is an open circuit unit that draws

conditioned room air into the chamber at the same flow rate as it is extracted into the gas analysis system. Each of the rooms is equipped with a toilet and sink with privacy screen, cycle ergometer, bed, desk, and computer with access to television and other forms of entertainment. Food and beverages are passed through an air-lock drawer system. Electrocardiogram telemetry and a nurse call are available to enhance subject safety. In the chamber, 24h energy expenditure (EE), sleeping EE, respiratory quotient (RQ), and the thermic effect of feeding (TEF) will be assessed.

**4.3 24-hour urine collection**

During each 24-hour respiratory chamber stay, all urine will be collected for measurement of total nitrogen, creatinine, catecholamines, and C-peptide.

**4.4 Doubly Labeled Water (DLW)**

On the morning beginning diet treatments 1 and 2, we will collect two baseline (predose) urine samples. These baseline urine samples will be collected prior to DLW dose. Each DLW dose will be obtained from a larger DLW batch containing a mixture of $^{2}H_2O$ (99% enrichment) and $H_2{}^{18}O$ (10% enrichment). The dose of DLW will be prepared individually for each subject (proposed dosage: 1 g DLW/kg of body weight).

After the baseline urine samples are collected, DLW will then be given to the subject to drink. After the subject has consumed the DLW, two rinses (each rinse is about 50 mL of tap water) will be added to the dosing container, and given to the subject to drink. Spot urine samples will be collected periodically during the subsequent 6 to 12 hours of the DLW dose administration and daily thereafter. The exact time of urine collection for each sample will be recorded. Isotopic enrichments of urine samples will be measured by isotope ratio mass spectrometry for the next 13 days. The average $CO_2$ production rate will be estimated from the differential disappearance of the 2 isotopes in the urine samples [20]. The energy expenditure calculations will use the average 24 hr RQ measurements from the respiratory chamber.

**4.5 Plasma substrate and hormone concentrations**

Blood samples will be collected into chilled test tubes containing 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 for isolation of plasma. After centrifugation, the plasma will immediately be frozen and stored for later analysis. Our current plans for measuring plasma substrate and hormone concentrations are described in the procedures outlined in Table 2.

**Table 2. Example list of procedures for measuring plasma substrates and hormones**

| | Metabolite/hormone | Proposed Method |
|---|---|---|
| **Metabolites** | Triglycerides | colorimetric assay |
| | Fatty acids | colorimetric assay |
| | Cholesterol | colorimetric assay |
| | Glucose | colorimetric assay |
| | Chem15 | |
| | Hemoglobin A1C | |
| **Hormones** | Insulin | RIA |

| | | |
|---|---|---|
| | Leptin | RIA |
| | C-peptide | RIA |
| | Adiponectin | RIA |
| | GLP-1 (active form) | RIA |
| | $PYY_{3-36}$ | RIA |
| | GIP | RIA |
| | Acyl- and total ghrelin | RIA |
| | FGF21 | ELISA |
| | TSH | CLIA |
| | Triiodothyronine (T3, f-T3) | CLIA |
| | Thyroxine (T4, f-T4) | CLIA |
| | Reverse-T3 | ELISA |
| **Markers of Inflammation** | CRP | ELISA |
| | IL-6 | ELISA |
| | IL-8 | ELISA |
| | TNF-alpha | ELISA |
| | E-selectin | ELISA |
| | sICAM-1 | FMIA |
| | sVCAM-1 | FMIA |
| | serum amyloid A | ELISA |

RIA = radioimmunoassay; HPLC = high performance liquid chromatography, ELISA = enzyme-linked immunosorbent assay; CLIA = chemiluminescence immunoassay; FMIA = fluorescent multiplexed bead-based assay

### 4.6 Questionnaires

*Profile of Mood States (POMS) Questionnaire:* The Profile of Mood States (POMS) is a widely used questionnaire for measuring distinct mood states. The POMS has 65 items, each with a 5-point adjective rating scale, which measures six identifiable moods or feelings: Tension-Anxiety (T), Depression-Dejection (D), Anger-Hostility (A), Vigor-Activity (V), Fatigue-Inertia (F) and Confusion-Bewilderment (C). Completion of the POMS takes 5-10 minutes.

*DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure:* 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. Cutoff scores, critical items, and follow-up measures are provided for each disorder. The questionnaire will be scored by a qualified member of the study team immediately after completion. If the subject scores above the cutoff for one or more disorders, these results will be discussed with a medically qualified team member, and a Clinical Center psychiatry consult will be obtained as appropriate.

*Food Frequency Questionnaires (FFQ):* Food Frequency Questionnaires (FFQ) are self-assessment questionnaires developed to identify foods and macronutrients that significantly contribute to energy intake [21]. 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 certain food items over a defined period of time.

*Three-Factor Eating Questionnaire:* The three-factor eating questionnaire (TEFQ) is a self-assessment questionnaire developed to measure dietary restraint, disinhibition and hunger [22]. 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.

*MacArthur Socioeconomic Status (SES) Questionnaire:* 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.

*Owl and Lark Sleep Questionnaire:* The Owl and Lark Sleep Questionnaire is a self-assessment questionnaire to determine morningness and eveningness in human circadian rhythms. The questionnaire contains 19 questions that require the subject to score based on wake and sleep habits.

*Sleep Apnea Clinical Score (SACS):* This questionnaire contains four items: measurement of neck circumference, history of hypertension, and two reported clinical symptoms (e.g. snoring, gasping during sleep). With these criteria, a sleep apnea clinical score (SACS) is generated, and a score greater than 15 indicates high risk for sleep apnea.

*STOP-Bang Questionnaire:* This questionnaire contains eight items, four of which are yes/no questions about snoring, tiredness, observed breathing during sleep, and blood pressure. The other four items are cut-off physical measurements and demographics (BMI, age, neck circumference, gender). Answering yes to three or more items indicates a high risk of OSA.

*Hunger & Satiety Assessment*: At several time points throughout the study, subjects will be asked to complete a survey to identify their perception of hunger (i.e., visual analog scale [VAS]) [22]. 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 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. On a few select days, data will be collected at least every 30 to 60 minutes over the next 2-3 hours after consuming each of their meals. It will take approximately 3 minutes for a participant to complete the assessment at a given time.

*Sensory and palatability assessments:* On several days throughout the study, subjects will be asked to complete a survey to assess the palatability and sensory properties (such as taste, texture, etc.) of the meals provided. Subjects will complete 100-point VAS line scale ratings of a series of measures, including meal liking and selected sensory properties. Survey items will be completed before eating, after the first bite, and after the participant has finished eating. Ratings include: how filling they expect a meal to be (expected fullness), how quickly or slowly they felt they ate the meal, and the extent to which they feel hungry, full and thirsty, their desire to eat and prospective consumption. The scale responses range from “Not at all” (0) to “Very” (100), except for prospective consumption, which is anchored by “Nothing at all” (0) and “The most I have ever eaten” (100). These ratings will be embedded amongst distracter “mood” ratings of alert, happy, and clear-headed. It will take approximately 3 minutes for a participant to complete the assessment at a given time.

**4.7 Dual energy x-ray absorptiometry (DXA)**

DXA scans will be performed at baseline followed by weekly scans 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 per scan is less than 1 mrem, and therefore the total of 5 scans planned per subject is below the guideline of 5000 mrem per year allowed for research subjects by the NIH Radiation Safety Committee. This is an FDA approved device that is being used as per approved labeling and parameters.

**4.8 Physical activity monitoring**

Physical activity will be quantified daily with an activity monitor using high sampling frequencies during all waking periods. Overall physical activity levels, daily changes, amount of time spent in sedentary, moderate, vigorous intensity categories and activity-associated energy expenditures will be extracted [23]. This device is an FDA cleared Class II medical device within the United States and is being used per approved labeling and parameters.

**4.9 Oral glucose tolerance test**

After an overnight fast, 75 grams of oral glucose will be administered. Blood samples will be obtained at 0, 10, 20, 30, 60, 90, 120, and 180 minutes to measure glucose and insulin concentrations. Blood samples for research will also be collected.

**4.10 Continuous Glucose Monitoring (CGM)**

Subjects will wear an FDA-approved CGM during their inpatient admission except during the MRS procedure. The device will be used to record glucose in real-time, 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 at least once every two weeks. This is an FDA approved device that is being used as per approved labeling and parameters.

**4.11 Sleep Monitor**

The sleep scanner is a wearable tracking device intended for monitoring sleep patterns, which may affect metabolism. The sleep scanner collects data on respiratory effort, oxygen saturation, pulse, snoring, body position, and movement. This device consists of a sensor board, a respiratory belt (respiratory impedance plethysmography), and oximetry probe. Subjects will apply the respiratory belt around the diaphragm and oximetry probe clips to their finger. Subjects will be asked to apply the device at bedtime for several nights during the study period. The wearable tracking device is synced to a computer and data will be retrieved for analysis.

**4.12 MRI/MRS**

The subject will lie supine in the MR gantry and will be asked to hold their breath during scans. The duration will depend on their tolerance. The MR imaging test will be performed in collaboration with another NIH investigator, Dr. Ahmed Gharib. Subjects will undergo the MRI/MRS after signing a separate informed consent document specific to the imaging study, protocol 11-DK-168 (Dr. Gharib's study). This separate imaging study consent also involves CT scan. A CT scan of your heart may be performed to confirm findings from our MRI study. However, the CT scan portion is optional and refusal to undergo CT scan will in no way affect participation in the current study. MR spectroscopy will be performed primarily for assessment of hepatic fat content. Each MRI scan will last no more than 2 hours.

Gadolinium, a contrast agent sometimes administered to enhance visualization of the heart and blood vessels, will not be used during MR study scans. The MRI used in this study is FDA approved and it is being used as per approved labeling and parameters.

**4.13 Saliva Collection**

Variation in basic taste receptor genes (sweet, salty, sour, bitter, and umami) and nutrient sensing genes may contribute to differences in food preferences and dietary patterns. The taste receptor genes and their variants will be chosen accordingly for sweet and umami (T1R [taste receptor, type 1]), bitter (T2R [taste receptor, type 2]), and salty (ENaC [epithelial sodium channel][24]). The saliva sample will be collected using Orangene® (Genotek, Kanata, Canada) and used for the isolation of genomic DNA. Genomic DNA samples will be used as a template in TaqMan® assays (Applied Biosystems, Foster City, CA) in duplicates. Genotyping will be done by investigators blind to any subject information. Samples will be stored in a -80 °C freezer. For genotype quality assurance, the concentration of DNA in stored samples will be checked using a Nanodrop® Spectrophometer ND100 prior to use. The observed genotypes will be checked for Hardy–Weinberg equilibrium. The exploratory analysis will investigate the relationship of taste and obesity-related genotype with dietary intake, and with sweet and salt preference and

thresholds. An analysis of variance will test whether taste detection thresholds or preference differ by genotype. Saliva collection takes approximately 2 minutes to complete. This procedure will be conducted at the beginning of the study period.

**4.14 Psychophysical Taste Task: Sucrose and Salt Detection Thresholds**

Sucrose and salt detection thresholds will be assessed using a two-alternative forced-choice staircase procedure was developed at the Monell Center for Adults [25-27]. The two-alternative forced choice is a psychophysical method developed to elicit responses about an individual's experience regarding a stimulus. It focuses on the evaluation of a single attribute (e.g., sweetness or saltiness), and the stimulus is adjusted based on the individual's responses [28, 29]. For this study, all testing will take place in a private, comfortable room. Subjects will be fasted for at least one hour before the task and acclimate to the testing room and to the researcher for approximately 15 minutes before testing. For the first trial and each subsequent trial, subjects will be presented with pairs of solutions in random order; within each pair, one solution will be distilled water, and the other will be the taste stimulus. Subjects will be instructed to taste the first solution presented within the pair, swish the solution in their mouth for 5 seconds, and expectorate. This will be repeated for the second solution within the pair. Between solutions, subjects will rinse their mouth with water; they will rinse once within a pair, and twice between successive pairs. After tasting both solutions within a pair, subjects will be asked to point to the solution that has a non-neutral taste. A tracking grid will be used to record subjects' responses [30-32]. This method eliminates the need for a verbal response and has been shown to be an effective method for assessing both taste and olfaction in children [30, 33]. The task takes approximately 30 minutes to complete. Participants will complete this task at the end of each test diet period.

**4.15 Psychophysical Taste Task: Sucrose and Salt Preference**

Sucrose and salt preference will be assessed using a two-series paired comparison-tracking method developed at the Monell Center for Adults [25-27]. Subjects will be presented with pairs of solutions differing in sucrose concentration (3, 6, 12, 24, and 36 g per 100 mL) and salt (0.92–6.14% wt/vol NaCl). They will be asked to taste the solutions 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 sucrose and salt concentrations chosen will be determined. This serves as an estimate of the participant's most preferred level of salt or sucrose [33, 34]. The task takes approximately 15 minutes to complete. Participants will complete this task at the end of each test diet period.

**4.16 Above-threshold or Suprathreshold sensory function**

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 (sucrose, NaCl) with the first "concentration" being water will be presented to the subjects. All four concentrations will be presented in random order without repeat. We will use 0.00, 0.09, 0.35, and 1.05 mol/l sucrose solutions and 0.00, 0.16, 0.38, and 1.05 mol/l salt solutions. Subjects will rate the perceived intensity of the stimulus using the gLMS scale and mean intensities of the two trials at each concentration for each stimulus to evaluate subjects' taste intensity perception.

The task takes approximately 10 minutes to complete. Participants will complete this task at the end of each test diet period.

**4.17 Slips-of Action Paradigm**

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 if subjects respond correctly to the cue box instructions they will receive a reward. For the second stage, no feedback will be provided for correct or incorrect responses. 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. The total task time will be 27 minutes. Participants may complete this computer based task during the inpatient admission, depending on time.

**4.18 Reward Prediction Error**

A computerized probabilistic procedural learning task, the Probabilistic Selection Task, to assess participants ability to learn from both positive and negative outcomes. 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). Both phases of the task combined last approximately 25 minutes. Participants may complete this computer based task during the inpatient admission, depending on time.

## 5. Statistical Analysis

Day to day variability of *ad libitum* energy intake has a standard deviation of about 500-600 kcal/d [35-37]. Therefore, over a 14-day diet period each subject will have a mean energy intake with a standard error of about 130-160 kcal/d and the mean energy intake difference between the study diets will have a standard error of about 190-230 kcal/d. Using the conservative assumption that within-subject energy intake correlations are zero, the 20-subject study cohort will have an 80% power to detect a difference in mean *ad libitum* energy intake between the diets of about 125-150 kcal/d with a Type I error probability of 0.05.

Previous *ad libitum* diet studies comparing mean energy intake between 14 day periods consuming diets differing in macronutrient content have observed differences >250 kcal/d [12, 13] which is greater than the minimum detectable difference for a 10-subject cohort under the same assumptions as above. Therefore, the 20-subject cohort of our study is adequately powered to detect physiologically significant differences in *ad libitum* mean energy intake between the diets.

## 6. Safety Considerations

### 6.1 Possible Risks and Hazards

Research-related risks in this study include those associated with study procedures, namely blood drawing, indirect calorimetry, respiratory chamber, doubly labeled water, measurement of body composition by dual energy x-ray absorptiometry (DXA), wearing physical activity monitors, continuous glucose monitoring, sleep monitoring, oral glucose tolerance testing, and measurement of liver fat by MRS. There are no study medications, and we do not anticipate any diet intervention-related risks since the study will be under close supervision with trained dietitians/nutrition experts.

*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. The approximate volume of blood to be drawn for research purposes is as follows:

**Research Blood Draws**

| Screening Visit | Diet 1 | Diet 2 | Total |
|---|---|---|---|
| 10 ml | 174 ml | 147 | 331 |

This is within the NIH guidelines for limits of blood drawn for research purposes (550 ml in any 8-week period).

*Oral Glucose Tolerance Test*. Subjects may experience faintness, nausea, and vomiting after taking the glucose solution.

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

*DXA.* The amount of radiation during the DXA scan is less than one mrem to the whole body. This radiation exposure is below the guideline of 5000 mrem per year allowed for research subjects by the NIH Radiation Safety Committee The use of the DXA scan apparatus may cause some minimal discomfort in claustrophobic subjects and may cause some minimal back pain in a small minority of the individuals.

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

*Respiratory chamber.* Besides inconveniences that can reasonably be expected as a result of spending an extensive time (24h) in the live-in room calorimeter, there is no risk to subjects' physical health. Claustrophobia is an exclusionary criterion. All subjects will be given an opportunity to experience the metabolic chamber prior to enrollment in the study.

*Profile of mood states questionnaire.* There is no known risk associated with the POMS questionnaire, however, there is the potential of subjects finding 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.

*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.

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

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

*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.

*Owl and Lark Sleep Questionnaire.* There is no known risk associated with the sleep self-assessment.

*Hunger and satiety assessment.* There are no known risks associated with the hunger and satiety assessment

*Sleep Apnea Clinical Score.* There is no known risk associated with the Sleep Apnea Clinical Score.

*STOP-Bang Questionnaire*. There is no known risk associated with the STOP-Bang Questionnaire.

*Sensory and palatability assessments.* There are no known risks associated with the sensory and palatability assessment.

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

*Continuous Glucose Monitoring System.* 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. Because this protocol represents the first use of this device by our research team, we do not know the frequency of such complications of the device. A medical provider will be available should any of these problems occur.

*Sleep Monitor.* Subjects may experience discomfort from the respiratory belt or oximetry probe. A plastic covering of the respiratory belt sensor board safeguards against direct skin contact with electrical parts and wiring. Participants will wear the device over thin clothing.

*MRI/MRS.* There is a small chance of claustrophobia or muscle-skeletal discomfort from lying partially in the magnet. During the imaging measurement, the noise may be somewhat unpleasant, but ear plugs will be provided for comfort. Although the long-term risk of exposure to a magnetic field is not known, the possibility of any long-term risk is extremely low from the information accumulated over the past ten years.
**MRS Warning: Certain implants, devices, or foreign objects implanted in the human body may interfere with the MR procedure. Volunteers who have undergone specific prior surgeries (i.e. heart, brain, gastric bypass, breast augmentation, etc) and/or have implants of specific types may be required to provide their IMPLANT CARD in order to determine implant safety/compatibility with the magnet before a scan is performed. The continuous glucose monitor will be removed prior to the MRS procedure.**

*Saliva collection for taste genotyping.* This is a non-invasive procedure and there are no risks associated with saliva collection. Information regarding genetic results will not be shared with the participants.

*Psychophysical taste task: Sucrose and Salt Detection Thresholds.* There are no risks associated with assessments of taste detection thresholds.

*Psychophysical taste task: Sucrose and Salt Preference.* There are no risks associated with assessments of taste preference.

***Above-threshold or Suprathreshold sensory function:*** There are no risks associated with assessments of taste preference.

***Slips of Action Paradigm:*** There are no risks associated with the Slip-of-Action paradigm assessment.

***Reward prediction error***: There are no risks associated with the Reward Prediction Error assessment.

**6.2 Risks Related to Clinical Relevance of Test Results**

If any lab tests, 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. If needed, the research team will refer subjects to a health care provider.

**6.3 NIH Reporting Requirement**

6.3.1 Adverse Event Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events.

6.3.2 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

6.3.3 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events.

6.3.4 Clinical Director Reporting

Problems expeditiously reported to the IRB in iRIS will also be reported to the Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

### 6.4 Data and Safety Monitoring Plan

Given the modest level of risk involved in the research, no Data and Safety Monitoring Board will be instituted. Medical oversight will be provided by the Medically Accountable Investigator. Adverse events will be recorded and monitored 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.

## 7. Recruitment Strategies

Adult subjects will be recruited from the community through advertisements in clinicaltrials.gov and possibly, local flyers, newspapers, magazines, internet, mail, radio, or television.

The age range for inclusion in this protocol is 18-50 years. The rationale for excluding subjects older than 50 years is multi-fold. First, 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). Second, 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.

### 7.1 Recruitment of Women, Children, Minorities and Other Vulnerable Individuals

NIH employees will be eligible to participate in this study. We will use appropriate methods to recruit staff participants, posting flyers where public announcements are permitted (rather than direct solicitation of subordinates). 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. 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. We expect women to represent approximately half of the study population, and we anticipate that African-Americans and Hispanics will constitute at least 20% of enrollees.

We have deliberately chosen not to study children and adolescents at this time. At present, there is no information suggesting that the metabolic responses to alterations of dietary macronutrients are likely different in adults and children and so we see no compelling reason to expose children to the rigors and inconvenience of this type of study. 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 child health. We have also chosen not to enroll pregnant women at this time, as there is no known prospect of direct benefit for the woman or the fetus, and we see no reason to expose either the woman or the fetus to the rigors and inconvenience of this type of study.

Individuals who are unable to provide consent will also not be enrolled. This is based on the strenuous demands of the study and the requirement to be able to read, understand and carefully follow study directions, and fill out several questionnaires throughout the study. We have chosen not to enroll non-English speaking participants, as these questionnaires are not currently available in languages other than English. We will make every effort to enroll minority men and women.

## 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. Subjects' decision not to participate or to withdraw from the study will not involve any penalty or loss of benefits to which they are entitled, and will not affect access to health care at the National Institutes of Health.

Also, there are several conditions that require the Principal/Co-Investigator to drop a study volunteer from this protocol, which include but are 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/Co-Investigator of the study site 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 will be kept confidential. 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) will be coded for storage in refrigerators and freezers in a locked

NIH laboratories. These samples will be stored indefinitely. The IRB will be notified in the event these samples are accidentally destroyed, lost or are anonymized.

Some clinically relevant research data will be stored indefinitely in the medical record and will be accessible to the subject for review by others of the participant's choosing (doctors, insurance companies etc.) after executing a release of information. This and other data will be maintained in databases, password protected and secure.

**9.1 Collaborations Using Stored Samples**

All stored samples and data that will be sent to collaborator/s will be stripped of subjects' personal identifiable information based on NIH guidelines. Any future collaborations requiring transfer of stored biological samples and data will be coded after informing the IRB and obtaining necessary assurances from the outside institution. The protocol will be amended and IRB approval will be sought when such collaborations are established. The following are the current collaborations on the study:

- Dr. Ciarán Forde at the National University of Singapore will receive data from sensory and palatability assessments of the diets.

- Dr. Michelle Cardel at the University of Florida will receive data from questionnaires and assessments about the diets as well as energy intake data to look at the effects of objective and subjectively measured social status and potential relationships to energy intake and diet quality.

- Dr. Tera Fazzino at the University of Kansas will receive data from questionnaires and assessments about the diets as well as energy intake data to test whether palatability may provide additional predictive utility for energy intake.

- Dr. Jeff Brunstrom at the University of Bristol of the United Kingdom will receive data regarding energy intake and macronutrient profile of meals to analyze whether differences in intake can be partially explained by the specific beverages in each meal and their role in modifying the overall energy density of the items consumed.

- Multiple collaborators at NCI will: (i) identify candidate biomarkers that discriminate between an ultra-processed and an unprocessed diet, (ii) identify a metabolomic profile of an ultra-processed diet, and (iii) identify candidate biomarkers of common UPF items and ingredients, such as dietary emulsifiers and high-fructose corn syrup.

- Multiple collaborators at NIAID will characterize effects on immune phenotype measured at the following levels:
    - Transcriptome using PBMC, or preferably, whole blood sampled in PAXgene tubes.
    - PBMC phenotype or phosphorylation responses, using mass cytometry measuring ~40 antigens.
    - Serum/plasma proteomics using SomaLogic to measure 1300 proteins.

## 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 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. 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).

## 11. Risk/Benefits to Study Participants

### 11.1 Known Potential Risks

Please refer to Section 6 "Safety Considerations" for the list of possible risks/hazards.

### 11.2 Known Potential Benefits

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 obesity, and that these insights may lead to new treatment options in the future. Abnormal values will be discussed with the study volunteers and forwarded to their primary care physicians with the subject's permission.

### 11.3 Assessment of Potential Risks and Benefits

Please refer to section 6 of the protocol.

## 12. Remuneration

Subjects will receive payment for the time and effort connected with the study according to the table below. Completion of the entire study needs to be strongly encouraged since incomplete data collection provides no scientific benefit despite introducing a small amount of risk to the subject. Therefore, we propose the following remuneration schedule:

- Total Reimbursement* for completing both diet treatments according to the table below:

* If early discharge occurs prior to study completion, total reimbursement will be at the rate of $40.00 per day, to include the day of discharge. This reimbursement rate goes into effect if the study is not completed for any reason, i.e. occurrence of illness, family emergency, or per investigator discretion in the event of noncompliance with study procedures. On a case-by-case

basis, adjustments may be made to the study schedule, including repeat testing to accommodate subjects who must leave the study temporarily in the event of an emergency.

The total remuneration for this study, if completed, is in accord with our previous study that included the same number of study days (09-DK-0081; $6520) and a similar level of inconvenience for the volunteers.

| Procedure | P | n | TOTAL |
|---|---|---|---|
| Daily Food Adherence | $60.00 | 28 | $1,680.00 |
| Inpatient per Diem | $40.00 | 28 | $1,120.00 |
| Body Composition (DXA) | $50.00 | 5 | $250.00 |
| Indirect Calorimetry with cart | $50.00 | 5 | $250.00 |
| Daily Weight and Accelerometer (per week) | $50.00 | 4 | $200.00 |
| Oral Glucose Tolerance Test (OGTT) | $100.00 | 2 | $200.00 |
| Doubly Labeled Water (DLW) | $50.00 | 2 | $100.00 |
| Questionnaires | $100.00 | 1 | $100.00 |
| 24hr Respiratory Chamber | $100.00 | 4 | $400.00 |
| MRS for Liver Fat | $100.00 | 3 | $300.00 |
| Continuous Glucose Monitoring (per week) | $100.00 | 4 | $400.00 |
| Sleep Monitoring | $50.00 | 4 | $200.00 |
| | **TOTAL REMUNERATION:** | | **$5,200.00** |

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# 14. Appendix A, Proposed Schedule

| Date → | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Processed/Unprocessed Diet | | | | | | | | | | | | | | | Unprocessed/Processed Diet | | | | | | | | | | | | | |
| 24 h energy expenditure (chamber) | | | | X | | | | | | | X | | | | | | | X | | | | | | | X | | | | |
| 24 h urine collection | | | | X | | | | | | | X | | | | | | | X | | | | | | | X | | | | |
| Liver fat content (MRS)[1] | | X | | | | | | | | | | | | | X | | | | | | | | | | | | | | X |
| DXA scan | | X | | | | | | X | | | | | | | X | | | | | | | X | | | | | | | X |
| REE/metabolic cart | | X[2] | | | | | | X | | | | | | | X | | | | | | | X | | | | | | | X |
| Fasted blood draw | | X | | | | | | | | | | | | | X | | | | | | | | | | | | | | X |
| Doubly labeled water dose | | X | | | | | | | | | | | | | | X | | | | | | | | | | | | | |
| DLW urine sample(s) | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Body weight | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Accelerometer | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Plex sleep monitor | | | | | | | | | | | | X | X | X | | | | | | | | | | | | | X | X | X | |
| Oral glucose tolerance test (OGTT) | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | X | |
| Hunger & satiety assessments | | | | | | | | | X | X | X | | | | | | | | | | | | X | X | X | | | | | |
| CGM sensor insertion/replacement | | X | | | | | | X | | | | | | | X | | | | | | | X | | | | | | | | |
| Pregnancy test (females) | | X[2] | | | | | | X | | | | | | | X | | | | | | | X | | | | | | | | X |
| Sensory & palatability assessments | | | | | | | | | | | | | | X | X | X | X | | | | | | | | | | | | | |
| Sucrose & salt detection | | | | | | | | | | | | | X | | | | | | | | | | | | | | | X | | |
| Sucrose & salt preference | | | | | | | | | | | | | | X | | | | | | | | | | | | | | | X | |
| Saliva collection | | | | | | | | | | | | | X | | | | | | | | | | | | | | | | | |

[1] MRS times: Mon PM, Tues PM, Thurs PM

[2] If needed