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

Date: October 28, 2020

PROTOCOL NUMBER: 19-DK-0067 **PROTOCOL VERSION:** 1.8

TITLE: Effect of Low Fat versus Low Carbohydrate Diets on Energy Metabolism

SHORT TITLE: Low-Carb vs. Low-Fat Diets

IDENTIFYING WORDS: Low Carbohydrate Diet, Low Fat Diet

TYPE OF PROTOCOL: Natural History – Disease Progression/Physiology

START DATE: March 2019 **END DATE:** December 2020

PRINCIPAL INVESTIGATOR:

Kevin Hall, Ph.D.*
Laboratory of Biological Modeling (LBM)
National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)
Building 12A, Room 4007
12 South Drive, (MSC 5621)
Bethesda, MD 20892-5621
Phone: 301-402-8248
Fax: 301-402-0535
E-mail: kevinh@niddk.nih.gov

NUMBER AND TYPE OF PATIENTS:

	Number	Sex	Age Range
Volunteers	Accrual ceiling = 200	Male and Female	18 – 50 years

PROJECT USES IONIZING RADIATION:
Yes Research indicated RSC Approval Number: Expiration Date: 2020

OFF-SITE PROJECT: No

MULTI-INSTITUTIONAL PROJECT: No

Table of Contents

Précis

Competing theories about obesity and its treatment contrast the relative roles of dietary fat versus carbohydrate on promotion of excessive calorie intake. Advocates of low-carbohydrate diets propose that diets high in carbohydrates lead to elevated insulin secretion and increased calorie intake. Alternatively, proponents of low-fat diets argue that diets high in fat promote passive overconsumption due to the high energy density and low satiety index of high-fat foods. Therefore, we will conduct a feeding study in 20 adult men and women to investigate the differences in *ad libitum* energy intake resulting from consuming two test diets for a pair of 2-week periods in a randomized, crossover design during a single 4-week inpatient period. The test diets presented to participants will be matched for calories and protein, but the low-carbohydrate diet (~10% of calories) will be high in fat (~75% of calories) whereas the low-fat diet will be high in carbohydrates (~75% of calories) and low in fat (~10% of calories).

1. Introduction and Background

Dietary fat contains more than twice the calories per gram compared to carbohydrate and protein. Furthermore, foods with high amounts of carbohydrates also typically have high water content thereby reducing their energy density and making them more satiating compared with high-fat foods [1, 2]. Therefore, high-fat diets are thought to promote excessive calorie intake and weight gain. However, dietary carbohydrates have also been suggested to a play causal role in the pathological accumulation of body fat [3-6]. According to the "carbohydrate-insulin model" of obesity, an increased proportion of the diet with high-carbohydrate foods results in elevated insulin secretion that suppresses the release of fatty acids into the circulation and directs circulating fat towards storage. This decreased availability of fatty acids for use by metabolically active tissues such as heart, muscle, and liver is perceived as a state of cellular "internal starvation", leading to an adaptive increase in calorie intake [3, 6-9].

While there is much debate surrounding the effect of diet composition on energy intake, most studies rely on self-reported measurements of diet which are known to provide inaccurate estimates of energy and macronutrient intake [10]. Furthermore, outpatient studies cannot ensure that subjects are exposed to only the diets under investigation and cannot adequately assess adherence. Only two previous studies have used objective methods to measure significant effects on *ad libitum* energy intake over 14-day periods between diets differing in fat (15-60% of total calories from fat) and diets differing in carbohydrate (27-67% of total calories from carbohydrate) [11, 12]. However, proponents of low-carbohydrate and low-fat diets often claim that the benefits of such diets require that the restricted macronutrient be limited to ≤ 15% of total calories. Furthermore, the previous *ad libitum* feeding studies employed 3-day rotating menus with identical foods in each test diet. Thus, the limited variety of foods raises questions about ecological validity since the types of foods typically consumed on a low-carbohydrate, high-fat (LCHF) diet often differ widely from the foods consumed on a high-carbohydrate, low-fat (HCLF) diet. For example, LCHF diets typically employ a variety of animal products and avoid foods high in sugar and starch. In contrast, LFHC diets are often plant-based with most calories derived from high-starch foods and the use of cooking fats and spreads is restricted.

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 a LCHF diet and a LFHC 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. The test diets 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.

This study will implement a pair of 7-day rotating menus for each test diet. The diets will be matched for presented calories and protein, but the LCHF diet will be low in carbohydrate (~10% of calories) and high in fat (~75% of calories) whereas the LFHC diet will be high in carbohydrates (~75% of calories) and low in fat (~10% of calories).The test diets will have a common foundation of vegetables with low amounts of digestible carbohydrates such as lettuce, spinach, kale, cabbage, cauliflower, zucchini, tomato, asparagus, broccoli, peppers, brussels sprouts, and green beans. The LCHF diet will add meat, poultry, fish, eggs, dairy, and nuts. The LFHC diet will add legumes, rice, root vegetables, soy products, corn, lentils, peas, whole grains, bread, and fruit. The diets will

be designed using a standard computerized nutrition database. Each subject will be admitted as an inpatient to the NIH Clinical Center and will stay on the 5SWN Metabolic Care Research Unit (MCRU).

Hypothesis: The hypothesis of this study is that the LCHF diet will lead to greater mean *ad libitum* energy intake as compared to a LFHC diet when the meals presented to the subjects are matched for daily calories and protein.

2.1 Primary Aims:

1. To determine differences in *ad libitum* energy intake (kcals) during 2 weeks of eating an LCHF diet as compared to 2 weeks of LFHC diet matched for presented calories and protein.
2. To determine differences in *ad libitum* energy intake (kcals) during the final week of eating a LCHF diet as compared to the final week of eating a LFHC diet matched for presented calories and protein.

The first primary aim maximizes the power to detect a significant effect whereas the second primary aim addresses the lack of an equilibration period upon transition to the first test diet from the pre-study period and the potential short-term carryover effect on the second test diet.

Previous studies have shown changes in glucose tolerance and insulin sensitivity after consuming diets that differ in their relative carbohydrate to fat content [13-18]. Hepatic triglyceride content can also be altered with isocaloric diet changes in as little as 9 days [19]. 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 a LCHF diet as compared to 2 weeks of consuming a LFHC diet matched for presented calories.
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 a LCHF diet as compared to 2 weeks of consuming a LFHC diet matched for presented calories and protein.
3. To determine changes in body weight and body composition after 2 weeks of consuming a LCHF diet as compared to 2 weeks of consuming a LFHC diet matched for presented calories and protein.
4. To determine changes in energy expenditure and substrate metabolism after 2 weeks of consuming a LCHF diet as compared to 2 weeks of consuming a LFHC diet matched for presented calories and protein.
5. To determine changes in hepatic triglyceride content after 2 weeks of consuming a LCHF diet as compared to 2 weeks of consuming a LFHC diet matched for presented calories and protein.

3. Study Design

This is a randomized order crossover study of healthy adult volunteers to determine differences in *ad libitum* energy intake when consuming a LCHF vs. LFHC diet matched for presented calories and protein.

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. To determine eligibility for the MRI component of the study, we will ask subjects about prior hearing loss that may be impacted by the MRI or metal implants such as pacemakers, metallic prostheses such as cochlear implants or heart valves, shrapnel fragments, etc.. All subjects will be fully informed of the nature, risks, and potential benefits of the study prior to giving written consent. However, subjects will not be explicitly told that the primary aims of the study are to measure differences in food intake between the test diets. Rather, they will be informed that the purpose of the study is to examine the health and metabolic effects of a low-carbohydrate, high-fat diet compared to a low-fat, high carbohydrate diet. Because it is not possible to blind subjects to the diets, concealing the primary aims of the study will limit the potential confound that subjects consciously alter their food intake behavior to purposefully increase or decrease their intake of the diet to influence the primary outcomes.

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) as determined by volunteer report
3. Body mass index (BMI) ≥ 20kg/m^2
4. Body weight ≥ 53 kg
5. 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. Positive pregnancy test or lactation as determined by volunteer report (women only)
4. Participating in a regular exercise program (> 2h/week of vigorous activity) as determined by volunteer report
5. Hematocrit <37% (women only)
6. Hematocrit < 40% (men only)
7. Caffeine consumption > 300 mg/day as determined by volunteer report

8. Regular use of alcohol (> 2 drinks per day), tobacco (smoking or chewing) amphetamines, cocaine, heroin, or marijuana over past 6 months as determined by volunteer report
9. Psychological conditions such as (but not limited to) eating disorders, 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 eating disorders as determined by volunteer report
    b. 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 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 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 to determined speed and resistance
- Food Frequency Questionnaire
- DSM-*5* Self-Rated Level 1 Cross-Cutting Symptom Measure [20]

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. The resting energy expenditure measurement is required to provide the nutrition department with the necessary information to determine the calorie level of the provided diets. Since volunteers may begin the study as soon as the day after screening, we will perform a REE measurement at the screening visit.

Volunteers will be asked to sample the diets during this visit. They will eat foods from the LCHF and LFHC 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 complete this study 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.

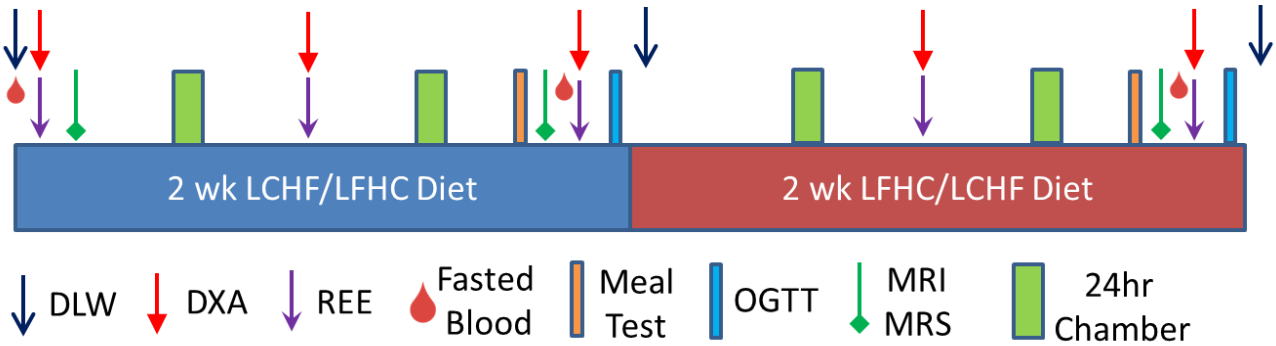

Figure 1. Study outline.

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
- Meal tolerance test
- Oral glucose tolerance test (OGTT)
- MacArthur Socio-Economic Status Questionnaire

- Three-Factor Eating Questionnaire
- Profile of Mood States Questionnaire

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. A meal tolerance test with a composition representative of the prevailing diet will be performed during each diet period. 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 pregnancy tests prior to DXA scans, and positive test results will preclude subjects from further participation.

Participants will consume an *ad libitum* amount of 3 meals per day plus snacks in the metabolic unit. After meal times, all meal trays will be collected, 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. Visitors will be allowed to meet with study subjects in a common area under observation of the nursing and/or research staff to avoid the exchange of food or beverages.

Subjects will be randomized to begin receiving one of two test diets with 7-day rotating menus, LCHF diet or a LFHC diet, for two weeks followed by the alternate diet for the subsequent two weeks. The daily meals presented to subjects will be matched for calories. All subjects assigned to the same diet will receive identical meals 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.

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 [21].

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

For five consecutive days during the second week of each diet period, including 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: 0.7 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 [22]. 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 1.

Table 1. 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	
	Homovallinic Acid	ELISA
	Hemoglobin A1C	
Hormones	Insulin	ELISA
	Leptin	ELISA
	C-peptide	ELISA
	Adiponectin	ELISA
	GLP-1 (active form)	ELISA
	PYY_{3-36}	ELISA
	GIP	ELISA
	Acyl- and total ghrelin	ELISA
	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

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

4.6 Questionnaires

Unless otherwise indicated, some of the assessments below may be completed multiple times across the participant's inpatient stay to assess any effect of diet.

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 "over the past week, including today": 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 [23]. 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 [24]. 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.

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.

Self Reported Habit Index. 12-item self-report instrument of habit strength (the Self- Report Habit Index. SRHI is a reliable and valid instrument. It measures habit strength by breaking it down into a number of features of habit; that is, history of repetition, automaticity (lack of control, lack of awareness, efficiency), and expression of one's identity.

Satisfaction with life, Happiness and Well Being scales. Satisfaction with Life Scale includes 5 items that assess general (e.g., my life is going well) aspects of subjective well-being.

*UPPS-P Impulsive Behavior Scale***:** 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.

Liking Survey: Subjects will complete a validated, 100-item liking survey comprised of foods, beverages, physical activities, sedentary activities, pleasant experiences, and unpleasant experiences. 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]

*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.

*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).

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.

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 [25].

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, insulin, C-peptide, free fatty acid, and lactate concentrations. Blood samples for research will also be collected. If the results of this test indicate an abnormal blood glucose level consistent with potential diagnosis of diabetes, the subject will be informed by a physician on the research team prior to being discharged from the study. However, such abnormal results may not be revealed until the end of the study to avoid influencing eating behavior and risk compromising the primary aim of the study. Note that the planned experimental manipulation of diet composition can impact glucose tolerance. Therefore, an abnormal glucose tolerance test in the present experimental context need not necessarily indicate that the subject has impaired glucose tolerance in the absence of the experimental dietary manipulations.

4.10 Meal test

After an overnight fast, a liquid meal will be provided matching the macronutrient content of the prevailing diet and amounting to 30% of the estimated daily calorie requirements for weight maintenance as determined by 1.6×REE measured at screening. Blood samples will be obtained at 0, 10, 20, 30, 60, 90, 120, 180, 240, 300, and 360 minutes to measure glucose, lactate, free fatty acid, triglyceride, C-peptide, and insulin concentrations. Blood samples for research will also be collected.

4.11 Continuous Glucose Monitoring (CGM)

Subjects will wear FDA-approved CGMs during their inpatient admission except during the MRS procedure. The devices 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. We will calibrate the device using fingerprick blood glucose meter measurements. After initial calibration, the system will require blood glucose calibrations twice a day. Sensors expire after 7 days, so a new one will be inserted at the beginning of each inpatient week.

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. Subjects will undergo the MRI/MRS after signing a separate informed consent document specific to the imaging study (protocol 11-DK-0168). This separate imaging study consent also involves CT scan. 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. Subjects with hearing loss that may be affected by the MRI or metal implants that are incompatible with MRI (e.g., pacemakers, metallic prostheses such as cochlear implants or heart valves, shrapnel fragments, etc.) will be advised to not participate in the MRI/MRS procedures.

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][26]). The saliva sample will be collected using Oragene® (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 end 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 [27-29]. 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 [30, 31]. 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 [32-34]. 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 [32, 35]. The task takes approximately 60 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 [27-29]. 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 [35, 36]. The task takes approximately 30minutes to complete. Participants will complete this task at the end of each test diet period.

4.16 Above-threshold or Suprathreshold intensity

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. 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. We will use 0.00, 0.09, 0.35, and 1.05 mol/l sucrose solutions and 0.00, 0.056, 0.18, and 0.56 mol/l salt solutions.

4.17 Computerized Behavioral Tasks

Some of the assessments below may be completed multiple times across the participant's inpatient stay to assess any effect of diet.

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.

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.

Liking and 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.

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.

4.18 Daily Ketone Measurements

We may measure daily blood levels of beta-hydroxybutyrate using a hand-held device with a small quantity of blood (about 20 μL) obtained by capillary sampling. We may also measure breath acetone levels using a handheld breathalyzer. Daily urine samples for ketone measurements may be collected. These measurements will allow for estimating the kinetics of onset and dissipation of nutritional ketosis when subjects transition to and from the low carbohydrate diet.

4.19 Fecal sampling for Microbiome Analyses

Fecal samples will be collected in sterile cryovials and will be stored at -80°C until DNA extraction during the admission. If available, fecal samples will be collected at least once during each diet period.

5. Statistical Analysis

Day to day variability of *ad libitum* energy intake has a standard deviation of about 500-600 kcal/d [37-39]. 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. Over the final 7-day diet period, each subject will have a mean energy intake with a standard error of about 190-230 kcal/d and the mean energy intake difference between the study diets will have a standard error of about 270-320 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 using the full 14-day period for each diet and about 175-210 kcal/d comparing the final 7 days of each diet period 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 [11, 12] 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, oral glucose tolerance testing, and measurement of liver fat by MRS. There are no study medications. A decrease in blood glucose is an expected outcome of eating a low carbohydrate diet and hypoglycemia is a possible risk, especially during the low-carbohydrate diet period. We will closely monitor all subjects throughout the study using twice daily fingerstick glucose measurements. If their blood glucose is <60 mg/dl and is associated with hypoglycemic symptoms (headache, dizziness, and shakiness) the Licensed Independent Practitioner (LIP) will be notified. Snacks will be given from the protocol snack bin and blood glucose will be checked in 15 minutes, and the procedure will be repeated until the blood glucose is 60 mg/dl or the participant is asymptomatic. If the blood glucose is <40 mg/dl, the test will be repeated and STAT blood glucose level will besent to the laboratory for verification . Fifteen grams of fast acting carbohydrates will be provided to the subject immediately and the process will be repeated every 15 minutes until the blood glucose level increases to 60 mg/dl or the symptoms subside. If hypoglycemia is recurrent or severe, we may choose to withdraw the participant from the study.

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 (including the screening visit) is 500 ml according to the schedule below.

Screening Visit	Diet 1	Diet 2	Total
10 ml	256 ml	244 ml	500 ml

This blood volume is within the NIH guidelines for limits of blood drawn for research purposes (550 ml in any 8-week period). Participants will be informed that they should not have other research blood sampling or provide a blood bank donation during any 8-week period that includes the study 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. Rapid administration of double-labeled water can rarely be associated with mild and transient dizziness. At the doses being administered in this protocol, this side effect is unlikely to occur.

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.

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

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

Self Reported Habit Index. There are no known risks associated with the sensory and palatability assessment.

Satisfaction with life, Happiness and Well Being scales. There are no known risks associated with the sensory and palatability assessment.

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

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

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

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

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.

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. There are no known clinical consequences of variations in the taste receptor genes that will be studied. 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 intensity: 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.

Liking and Wanting: There are no risks associated with the Liking and Wanting assessment.

Delay Discounting: There are no risks associated with the Delay Discounting assessment.

Daily ketone measurements: 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. Blood ketones are expected to be low ≤0.6 mmol/L but may be higher (up to 1.5mmol/L) under fasting conditions, during exercise vigorously or when the person is ill. If a person is ill and has a blood ketone measurement of >1.5mmol/L, a confirmatory blood sample will be sent to the NIH CC lab and the medical provider notified.

Stool Sampling: Stool sampling is not associated with any health risk but may be uncomfortable for some participants.

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 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.

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. Investigator Responsibilities

The Principal 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. Michael Stagliano, CRNP will oversee pre-screening, participant management and admission, physical health assessments, and data acquisition and analysis. Juen Guo, PhD will assist in data analysis. Dr. Paule Joseph will assist with psychophysical testing and taste genotyping. Dr. Ciaran Forde will assist with sensory and palatability assessments.

8. 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. Advertisements will be developed with the assistance of the patient recruitment office and will be submitted to the IRB prior to their use.

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 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.

8.1 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.

9. 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. We will employ a recruit to replace strategy to accrue a total of 20 completers. 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

10. 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 and may be used for future research. 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.

10.1 Collaborations Using Stored Samples

Dr. Ciarán Forde at the National University of Singapore will receive data from sensory and palatability assessments of the diets. Dr. John Speakman at the University of Aberdeen will receive urine samples for metabolomics analyses and fecal samples for DNA extraction to reconstruct food intakes. Ethan Weiss of Keyto, Inc. will provide breath sensor devices and associated software and hardware to measure breath ketones.

Other collaborations will entail (i) identifying candidate biomarkers that discriminate between a low-carbohydrate, high-fat diet and a low-fat, high carbohydrate diet, (ii) identifying a metabolomic profile of a low-carbohydrate, high-fat diet and a low-fat, high carbohydrate diet, (iii) identifying candidate biomarkers of common UPF items and ingredients, such as dietary emulsifiers and high-fructose corn syrup, and (iv) conducting new lipoprotein analyses on stored plasma samples that were originally collected during the mixed meal tests . In addition, characterizing effects on immune phenotypes will take place, measured at the following levels: i) Transcriptome using PBMC, or preferably, whole blood sampled in PAXgene tubes, ii) PBMC phenotype or phosphorylation responses, using mass cytometry measuring ~40 antigens, and iii) Serum/plasma proteomics using SomaLogic to measure 1300 proteins.

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.

11. 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. Non-English speaking subjects are excluded from the study, therefore the use of the short form consent will not be required. The consent process will additionally be documented in the electronic medical record (CRIS). Principal Investigator, Dr. Kevin Hall, PhD, and Associate Investigator, Andrew Bremer, M.D., Ph.D., Stephanie Chung, M.B.B.S., Valerie Darcey, Ph.D., R.D., Irene Rozga, B.S.N.-R.N. and Michael Stagliano, CRNP will obtain the informed consent.

12. Risk/Benefits to Study Participants

This research study involves minimal risk related to the research-indicated procedures. 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.

13. 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
Meal test	$100.00	2	$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
	TOTAL REMUNERATION:		**$5,200.00**

14. References

1. Bray, G.A. and B.M. Popkin, *Dietary fat intake does affect obesity!* Am J Clin Nutr, 1998. **68**(6): p. 1157-73.
2. Stubbs, R.J., *Nutrition Society Medal Lecture. Appetite, feeding behaviour and energy balance in human subjects.* Proc Nutr Soc, 1998. **57**(3): p. 341-56.
3. Ludwig, D.S. and M.I. Friedman, *Increasing adiposity: consequence or cause of overeating?* JAMA, 2014. **311**(21): p. 2167-8.
4. Lustig, R.H., *Childhood obesity: behavioral aberration or biochemical drive? Reinterpreting the First Law of Thermodynamics.* Nat Clin Pract Endocrinol Metab, 2006. **2**(8): p. 447-58.
5. Taubes, G., *The science of obesity: what do we really know about what makes us fat? An essay by Gary Taubes.* BMJ, 2013. **346**: p. f1050.
6. Wells, J.C. and M. Siervo, *Obesity and energy balance: is the tail wagging the dog?* Eur J Clin Nutr, 2011. **65**(11): p. 1173-89.
7. Astwood, E.B., *The heritage of corpulence.* Endocrinology, 1962. **71**: p. 337-41.
8. Pennington, A.W., *Obesity.* Med Times, 1952. **80**(7): p. 389-98.
9. Pennington, A.W., *A reorientation on obesity.* N Engl J Med, 1953. **248**(23): p. 959-64.
10. Winkler, J.T., *The fundamental flaw in obesity research.* Obes Rev, 2005. **6**(3): p. 199-202.
11. Lissner, L., et al., *Dietary fat and the regulation of energy intake in human subjects.* Am J Clin Nutr, 1987. **46**(6): p. 886-92.
12. Stubbs, R.J., et al., *Covert manipulation of the ratio of dietary fat to carbohydrate and energy density: effect on food intake and energy balance in free-living men eating ad libitum.* Am J Clin Nutr, 1995. **62**(2): p. 330-7.
13. Himsworth, H.P., *The dietetic factor determining the glucose tolerance and sensitivity to insulin in healthy men.* Clinical Science, 1935. **2**: p. 67-94.
14. Numao, S., et al., *Short-term low carbohydrate/high-fat diet intake increases postprandial plasma glucose and glucagon-like peptide-1 levels during an oral glucose tolerance test in healthy men.* Eur J Clin Nutr, 2012. **66**(8): p. 926-31.
15. Numao, S., et al., *Effects of a single bout of aerobic exercise on short-term low-carbohydrate/high-fat intake-induced postprandial glucose metabolism during an oral glucose tolerance test.* Metabolism, 2013. **62**(10): p. 1406-15.
16. Pehleman, T.L., et al., *Enzymatic regulation of glucose disposal in human skeletal muscle after a high-fat, low-carbohydrate diet.* J Appl Physiol (1985), 2005. **98**(1): p. 100-7.

17. Sweeney, J., *Dietary factors that influence the dextrose tolerance test: A preliminary study.* Archives of Internal Medicine, 1927. **40**(6): p. 818-830.
18. Swinburn, B.A., et al., *Deterioration in carbohydrate metabolism and lipoprotein changes induced by modern, high fat diet in Pima Indians and Caucasians.* J Clin Endocrinol Metab, 1991. **73**(1): p. 156-65.
19. Schwarz, J.M., et al., *Effects of Dietary Fructose Restriction on Liver Fat, De Novo Lipogenesis, and Insulin Kinetics in Children With Obesity.* Gastroenterology, 2017.
20. Association, A.P. *DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure—Adult*. 2013; 2013:[Available from: https://www.psychiatry.org/psychiatrists/practice/dsm/educational-resources/assessment-measures.
21. Weir, J.B.d.V., *New methods for calculating metabolic rate with special reference to protein metabolism.* J Physiol, 1949. **109**(1-2): p. 1-9.
22. Schoeller, D.A., et al., *Energy expenditure by doubly labeled water: validation in humans and proposed calculation.* Am J Physiol Regul Integr Comp Physiol, 1986. **250**(5): p. R823-830.
23. Champagne, C.M., et al., *Dietary intakes associated with successful weight loss and maintenance during the Weight Loss Maintenance trial.* J Am Diet Assoc. **111**(12): p. 1826-35.
24. Stunkard, A.J. and S. Messick, *The three-factor eating questionnaire to measure dietary restraint, disinhibition and hunger*. Journal of Psychosomatic Research, 1985. **29**(1): p. 71-83.
25. Chen, K.Y. and D.R.J. Bassett, *The technology of accelerometry-based activity monitors: current and future.* Med Sci Sports Exerc, 2005. **37**(11 Suppl): p. S490-500.
26. Bachmanov, A.A., et al., *Genetics of taste receptors.* Curr Pharm Des, 2014. **20**(16): p. 2669-83.
27. Cowart, B. and G. Beauchamp, *Early development of taste perception.* Psychological basis of sensory evaluation, 1990: p. 1-17.
28. Mennella, J.A., et al., *Evaluation of the Monell forced-choice, paired-comparison tracking procedure for determining sweet taste preferences across the lifespan.* Chem Senses, 2011. **36**(4): p. 345-55.
29. Pepino, M.Y. and J.A. Mennella, *Effects of cigarette smoking and family history of alcoholism on sweet taste perception and food cravings in women.* Alcohol Clin Exp Res, 2007. **31**(11): p. 1891-9.
30. Bartoshuk, L.M., *The psychophysics of taste.* Am J Clin Nutr, 1978. **31**(6): p. 1068-77.
31. Jogan, M. and A.A. Stocker, *A new two-alternative forced choice method for the unbiased characterization of perceptual bias and discriminability.* J Vis, 2014. **14**(3): p. 20.
32. Bobowski, N.K. and J.A. Mennella, *Disruption in the Relationship between Blood Pressure and Salty Taste Thresholds among Overweight and Obese Children.* J Acad Nutr Diet, 2015. **115**(8): p. 1272-82.
33. Joseph, P.V., D.R. Reed, and J.A. Mennella, *Individual Differences Among Children in Sucrose Detection Thresholds: Relationship With Age, Gender, and Bitter Taste Genotype.* Nurs Res, 2016. **65**(1): p. 3-12.

34. Pepino, M.Y., et al., *Obese women have lower monosodium glutamate taste sensitivity and prefer higher concentrations than do normal-weight women.* Obesity (Silver Spring), 2010. **18**(5): p. 959-65.
35. Mennella, J.A., et al., *Preferences for salty and sweet tastes are elevated and related to each other during childhood.* PLoS One, 2014. **9**(3): p. e92201.
36. Mennella, J.A. and N.K. Bobowski, *Psychophysical Tracking Method to Measure Taste Preferences in Children and Adults.* J Vis Exp, 2016(113).
37. Tarasuk, V. and G.H. Beaton, *Day-to-day variation in energy and nutrient intake: evidence of individuality in eating behaviour?* Appetite, 1992. **18**(1): p. 43-54.
38. Bray, G.A., et al., *Corrective responses in human food intake identified from an analysis of 7-d food-intake records.* Am J Clin Nutr, 2008. **88**(6): p. 1504-10.
39. Edholm, O.G., et al., *Food intake and energy expenditure of army recruits.* Br J Nutr, 1970. **24**(4): p. 1091-107.

15. 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
	LCHF/LFHC Diet															LFHC/LCHF Diet													
24 h energy expenditure (chamber)				X							X							X							X			
24 h urine collection				X					X	X	X	X	X					X					X	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
Urine metabolomic analysis (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
Ketone urine (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
Ketone breathalyzer		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
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
Meal test												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