Study Protocol Title:
The Influence of Glycemic Control and Obesity on Energy Balance and Metabolic Flexibility in Type 1 Diabetes.

Study Sponsor:
Florida Hospital

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List of Abbreviations:

ACT1ON- Advancing Care for Type 1 Diabetes Obesity Network
AE- Adverse event
ALT - Alanine aminotransferase
AST - Aspartate aminotransferase
BIS-15 - Barratt Impulsiveness Scale
BMI - Body mass index
BP - Blood Pressure
CAP™ - Controlled Attenuation Parameter
CBC - Complete Blood count
CES-D - Center for Epidemiological Studies-Depression
CGM – Continuous glucose monitoring
CITI - Collaborative Institutional Training Initiative
CMP - Comprehensive Metabolic Panel
Conmed- Concomitant medication
Co-PI - Co- Principal Investigator
CRF - Case Report Forms
CRU – Clinical Research Unit
DCCT – Diabetes Control and Complications Trial
DEPS-R - Diabetes Eating Problem Survey
DEXA – Dual-Energy X-ray Absorptiometry
DI - Diabetes Institute
DKA – diabetic ketoacidosis
DSMB - Data and Safety Monitoring Board
EE – Energy Expenditure
EKG - Electrocardiogram
FDA- Food and Drug Administration
FH - Florida Hospital
FISMA - Federal Information Security Management Act
GCP- Good Clinical Practices
GFR - Glomerular filtration rate
GI- Gastrointestinal
HIPPA - Health Insurance Portability and Accountability Act
ICH- International Conference on Harmonization
IHL - Intrahepatic Lipid
IRB - Institutional Review Board
jMRUI - Java-Based Magnetic Resonance User Interface
kcal - kilogram calorie
kPa - kilopascals
LDL - Low density lipoprotein
LSM - Liver stiffness measurement
MFC - Mass flow controllers
mmol/L – millimoles per liter
MPI - Multi Principal Investigator
MRE - Magnetic Resonance Elastography

MRI - Magnetic Resonance Imaging
MRS - Magnetic Resonance Spectroscopy
NCT – ClinicalTrials.gov registry number
NDSR - Nutrient Data System for Research
NIDDK - National Institute of Diabetes and Digestive and Kidney Diseases
NIH - National Institute of Health
PDFF- Proton Density Fat Fraction
PDPAR - Physical Activity Recall
PHI - Protected Health Information
PI - Principal Investigator
PID -Participant Identifier
PRESS - point resolved spectroscopy
QBC - quadrature body coil
RD - Registered Dietician
rDNA- Recombinant Deoxyribonucleic Acid
RNA-seq - RNA sequencing
RPAQ - Recent Physical Activity Questionnaire
RQ Respiratory Quotient
SBPMDI - Sanford Burnham Prebys Medical Discovery Institute
SDV - Source Data Verification
SIRS - Sheps Integrated Research System
SOP - Standard operating procedures
SSL - Secure Sockets Layer
SSP - Study Specific Procedures
T1D – Type 1 Diabetes
THC - Tetrahydrocannabinol
TRI-MD – Translational Research Institute for Metabolism and Diabetes
TSH- Thyroid stimulating hormone
UNC - University of North Carolina
UNC- NORC - University of North Carolina Nutrition Obesity Research Center
USDA - United States Department of Agriculture
VCH_4 – Methane production
VCO_2 – Carbon dioxide production
VCTE™ - vibration-controlled transient elastography
VO_2 – Oxygen consumption

Introduction

This document describes a clinical research protocol to investigate the influence of glycemic control and obesity on energy balance and metabolic flexibility in T1D. The study will be conducted in compliance with the protocol, Good Clinical Practices (GCP) International Conference on Harmonization (ICH) Guidelines (E6) for GCPs standards as adopted by the Food and Drug Administration (FDA) and associated Federal regulations, and all applicable institutional research requirements.

Note: Minor modifications have been made to this protocol compared to the original grant with respect to the flow of events and number of visits (particularly, the addition of an extra inpatient visit for metabolic acclimatization). We also removed some procedures due to budget cuts: 4-Hour Mixed MEAL Test and Ad-Libitum Buffet Test Meal. In addition to the portion funded by the NIH (Phase 1), this IRB protocol includes a second set of studies (Phase 2) that will be funded by internal funds. We also added procedures to Phase 1 that are not included in the original grant and will be covered by internal funds: 1) FibroScan/MRE/Whole Body MR for acquiring more in-depth data on metabolism and fat deposition and 2) Stool collection for future analysis of the gut microbiome. This IRB protocol covers only the aim of the grant that will be completed at TRI (Aim 1, Study 1).

Background Information and Scientific Rationale

There has been an alarming increase in the prevalence of obesity in type 1 diabetes (T1D) in recent years. Among young adults with T1D, recent data indicate that 31% are overweight and 15% are obese [1]. Consistent with longitudinal studies that have shown that obesity increases in people with T1D as they age, other studies have shown that the prevalence of overweight and obesity exceeds 50% in adults with T1D [1-4]. Thus, the prevalence of overweight and obesity in patients with T1D now approaches that of the general population.

While some of the increase in obesity in patients with T1D is likely due to temporal trends in diet and physical activity affecting the population at large, it is probable that the widespread adoption of intensive insulin therapy for the treatment of T1D has also contributed to the increases in obesity in this population. The US Diabetes Control and Complications Trial (DCCT) demonstrated that intensive insulin therapy significantly decreased risk for microvascular complications, but also showed that this intervention was associated with significant weight gain [5]. Emerging evidence suggests that obesity contributes to insulin resistance, dyslipidemia and cardiometabolic complications in T1D [6,7]. For example, participants in the DCCT intensive treatment arm who experienced excessive weight gain manifested a worse cardiometabolic profile [6].

Young adults with T1D, in particular, appear to struggle to manage the competing priorities of glycemic control and optimal weight. Recent T1D Exchange data demonstrate age-dependent increases in HbA1c, with a peak mean of over 9% in 17-year-olds, which remains elevated above 8% until age 30 y [8]. Some patients with T1D omit insulin or purposefully deliver less insulin than is needed to avoid weight gain [9]. Uncontrolled diabetes causes glucosuria and an increase in metabolic rate [10]. These adaptations promote weight loss at the expense of higher HbA1c levels and an increased risk of complications.

The American Diabetes Association recommends that for “overweight or obese adults with type 2 diabetes, reducing energy intake while maintaining a healthful eating pattern is recommended to promote weight loss” but does not address obesity management for T1D [11]. Standard equations for estimating energy expenditure and dietary requirements in healthy individuals are not adequate for those with T1D. First, in patients with uncontrolled T1D, glycosuria can account for 300-400 kcal/day in obligate energy losses [5]. Second, most studies demonstrate a modest increase in resting energy expenditure (REE; range=100-300 kcal/day) in people with T1D relative to healthy

controls or predicted values [5-9]. Finally, there are virtually no prospective studies of obesity treatment in patients with T1D on which to base recommendations.

There is, thus, an urgent need for specific, evidence-based recommendations for the prevention and management of obesity in T1D aimed at optimizing not just one, but two key outcomes; glycemic control and weight status. To facilitate the development of these recommendations, we propose to develop a quantitative model of energy balance in young adults with T1D, accounting for differences in sex, glycemic control and body composition. The model will address energy requirements in T1D and provide preliminary data on variability in macronutrient metabolism. This information is important for designing interventions that are better matched to metabolic needs and, thus, are more likely to lead to successful control of blood glucose and weight. Further, we will pilot a dietary intervention to promote weight loss and improved glycemic control.

Study Objectives

Primary Objective/Aim/Goal/Hypothesis

The **Primary Objective** of this study is to develop a comprehensive model of energy balance and metabolic flexibility for T1D that accounts for the degree of hyperglycemia and differences in body composition.

To address this aim, we will enroll 33 young adults 19-30 years of age (8 lean T1D, 8 overweight T1D, and 8 obese T1D, with 3 sex- and weight-matched non-diabetic controls for each group), measure body composition (DEXA and whole-body MRI) and measure 24-hour energy expenditure and substrate oxidation rates in the respiratory chambers at the Translational Research Institute for Metabolism and Diabetes (TRI-MD).

Secondary Objective/Aim/Goal/Hypothesis

The **Secondary Objective** of this research protocol is to: Perform a pilot study of the safety, tolerability and metabolic effects of a 4-week low-carbohydrate calorie-restricted diet intervention on glycemic control, body weight, liver fat and volume, body composition and energy expenditure on overweight and obese subjects.

Study Design

Research Design

This is a non-randomized, cross sectional study comparing the metabolic phenotype of a range of body weights in individuals with and without T1D. We will assess the same parameters only in overweight and obese individuals to T1D after 1 month of a low carbohydrate, reduced calorie diet. This research protocol consists of 2 phases.

Phase 1: This phase of this research protocol consists of a cross-sectional study in 24 weight-stable young adults (~12 males, 12 females; 19-30 years of age) with T1D of at least 1-year duration and a range of glycemic control (A1c 6.5-13%) and age (also 19-30 years of age), sex, and BMI (+/- 3 kg/m^2) matched non-diabetic controls (total n=33). Participants will be divided equally among 3 groups: lean (BMI 18-24.9 kg/m^2), overweight (BMI 25-29.9 kg/m^2), and obese (BMI 30-39.9 kg/m^2). Participants with T1D can be on any insulin delivery method for their medical care and will remain on their own program throughout the study. Participants will be admitted to the TRI

Clinical Research Unit (CRU) for 4 days (3 nights) for a comprehensive metabolic assessment, including measurement of body composition by Dual Energy X-ray Absorptiometry (DEXA) and whole-body MRI, continuous glucose monitoring (CGM), and 24-hour energy expenditure and substrate oxidation rates in whole room calorimeters with simultaneous measurement of 24-hour urinary glucose excretion. From these data we will develop a comprehensive model of energy balance for young adults with T1D that accounts for clinically relevant factors including sex, glycemia, insulin use and body composition.

Phase 2: This phase consists of a 4-week low-carbohydrate calorie-restricted dietary intervention on glycemic control, body weight, body composition and energy expenditure. Overweight and obese participants with T1D from Phase 1 (n = 10) will be asked to alter their diet under the guidance of a nutritionist or registered dietitian (RD) and the medical team over a 4-week period (3 weeks outpatient self-selected, 5 days outpatient with provided meals and 2 days inpatient). After approximately 4 weeks on this diet, participants will return to the TRI-MD for re-evaluation using a protocol identical to that employed at baseline (Phase 1).

Study Agent, Device, and/or Intervention Description

The Phase 2 diet prescription will have a 500 kcal/day calorie deficit and will contain approximately 14% of calories from carbohydrate. Participants/Study Staff will perform continuous glucose monitoring via CGM or Point of Care (POC) blood glucose for the entire period so that insulin can be adjusted as necessary to prevent hypoglycemia. They will meet weekly with a nutritionist or a registered dietitian either in person or by phone and have their insulin adjusted on a daily basis or as needed in consultation with the PI and the medical staff of the TRI.

Twenty-four hour heart rate monitoring will be performed simultaneously with energy expenditure measurements in the chamber to allow calibration and refinement of the model for free-living energy expenditure we will derive in patients with type 1 diabetes. As such, this data is not a primary or secondary endpoint, but is considered exploratory. Heart rate data from wearable devices could then be used, along with age, sex, HbA1c and estimates of body composition to provide more accurate estimates of energy requirements for dietary interventions, for example. There are no safety issues that require heart rate monitoring in this particular population or study. We do not anticipate any output from this monitoring that would require real-time monitoring, consequently, we will only use the device to capture data for subsequent downloading.

Study Site(s)/Location(s) and Number of Subjects

This study will be conducted by Investigators at the Florida Hospital Translational Research Institute for Metabolism and Diabetes (TRI-MD). All study visits will take place at the TRI-MD facility. This study represents one aim of a multicenter NIH grant awarded to UNC Chapel Hill on which Dr. Pratley is a PI and Dr. Corbin is a Co- PI.

Estimated number of subjects enrolled at TRI-MD:

33 weight-stable young adult participants.

Name of external site(s) outside of Florida Hospital:

N/A

Estimated number of subjects at external sites:
0

Total number of all sites:
1

Estimated number of subjects at all sites combined:
33

Multi-Site Research Logistics/Communication Plan

Florida Hospital TRI-MD is the single clinical site enrolling participants.

Florida Hospital is sharing samples and data with other investigators.

- All other sites conducting work on samples collected during the clinical study will have the most current version of the protocol, consent document, and HIPAA authorization.
- All required approvals will have been obtained at each site (including approval by the site's IRB of record).
- All modifications will have been communicated to sites, and approved (including approval by the site's IRB of record) before the modification is implemented.
- All engaged participating sites will safeguard data as required by local information security policies.
- All local site investigators will conduct the study appropriately.
- Any non-compliance with the study protocol or applicable requirements will be reported in accordance with local policy.

Describe the method for communicating to participating sites:

- Problems - will be handled as per the attached MPI plan as submitted to NIH
- The closure of a study - will be handled as per the attached MPI plan as submitted to NIH

Research Conducted in a Foreign Country

Not Applicable

Community-Based Participatory Research

Not Applicable

Subject Selection

Vulnerable Populations

Florida Hospital (FH) Employees: Recruitment efforts will follow FH recruitment Standard Operating Procedures (SOPs) for research. FH Employees will not be individually targeted nor excluded from study participation based on employment. FH employees who engage the TRI-MD asking to participate in the study will be processed per standard consent procedures for participants. In addition, during the consent process, the study staff will review standard consent language stating that an employee's participation or lack of participation in the study will not affect their employment status or relationship with Florida Hospital.

Cognitively impaired individuals, children or people incarcerated in a correction facility will not be recruited.

Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for participation:

1. Males and females, 19 to 30 years of age, inclusive.
2. Type 1 Diabetes Cohort:
    a. Diagnosis of type 1 diabetes for greater than 1 year at screening.
    b. Hemoglobin A1c 6.5-13%

    or

    Non-Diabetes Control Cohort:
    a. Healthy individuals (non-diabetic) matched to T1D cohort by BMI, and gender
3. Able to provide informed consent.
4. BMI 18-39.9

Exclusion Criteria

1. Type 2 diabetes
2. History or presence of cardiovascular disease (unstable angina, myocardial infarction or coronary revascularization within 6 months, clinically significant abnormalities on EKG, presence of cardiac pacemaker, implanted cardiac defibrillator)
3. Liver disease (AST or ALT >2.5 times the upper limit of normal), history of hepatitis
4. Kidney disease (creatinine >1.6 mg/dl or estimated GFR<60 ml/min)
5. Triglycerides >800 mg/dl, LDL >200 mg/dl
6. Anemia (hemoglobin <12 g/dl in men, <11 g/dl in women)
7. Thyroid dysfunction. Participants with a TSH > 10 μ IU or less than 0.4 μ IU are excluded. Participants on thyroid replacement medication may be enrolled providing they have been on a stable dose of medication for at least 6 weeks prior to screening and their TSH is within the range specified above.
8. Uncontrolled hypertension (BP >160 mmHg systolic or > 100mmHg diastolic)
9. History of cancer within the last 5 years (skin cancers, with the exception of melanoma, may be acceptable).
10. Initiation or change in hormone replacement therapy within the past 3 months (including, but not limited to thyroid hormone, birth control or estrogen replacement therapy)
11. History of organ transplant
12. History of HIV, active Hepatitis B or C, or Tuberculosis

13. Pregnancy, lactation or 6 months postpartum from screening visit
14. History of major depression
15. Psychiatric disease prohibiting adherence to study protocol
16. History of eating disorders
17. Cushing's disease or syndrome
18. History of bariatric surgery
19. Tobacco use within the past 3 months
20. History of drug or alcohol abuse (≥3 drinks per day) within the last 5 years, except positive Tetrahydrocannabinol (THC) is acceptable.
21. Use of oral or injectable anti-hyperglycemic agents (except insulin)
22. Current use of beta-adrenergic blocking agents
23. Drugs that affect immune, weight or metabolic function, including but not limited to: oral corticosteroids, oral or injectable anti-obesity medications, oral or injectable antihyperglycemic medications other than insulin within 3 months of screening. Drugs for dyslipidemia (statins, ezetimibe, etc) and a daily full strength or baby aspirin for atherosclerosis prevention will be allowed, provided patients have been on stable doses for at least 6 weeks prior to screening.
24. Use of antibiotics within the past 3 months
25. Weight >450 lbs. (This is DEXA table weight limit)
26. Metal implants (pace-maker, aneurysm clips) based on Investigator's judgment at screening
27. Unable to participate in MRI or MRS assessment based on Investigator's judgment at screening
28. Participants with strict dietary concerns (e.g. vegetarian or kosher diet, multiple food allergies, or allergies to food we will provide them during the study)
29. Gastrointestinal disorders including: inflammatory bowel disease or malabsorption, swallowing disorders, suspected or known strictures, fistulas or physiological/mechanical GI obstruction, history of gastrointestinal surgery, Crohn's disease or diverticulitis.
30. Presence of any condition that, in the opinion of the investigator, compromises participant safety or data integrity or the participant's ability to complete study visits

Resources Available

We attest that all TRI faculty and staff will be trained and this training will be documented. We will adhere to FH ORA SOP 06 (Research Personnel Selection, Qualification, Responsibilities, and Training) and POLICY-TRI-QM-002 (Training Policy).

Study Procedures

Subject Recruitment and Screening

Subjects will be identified by physician referrals from the patient database of the Florida Hospital Diabetes Institute, as well as the database of the Translational Research Institute. Recruitment methods utilized may include, but will not be limited to the following: advertising in multiple media such as print ads, flyers, brochures, posters and social media advertisements and/or postcards will also be used to reach out to potential subjects.

All advertising materials will be submitted to the IRB for review prior to using or publishing them. A database search via Florida Hospital's Medical Record may also be utilized to identify potential candidates.

Recruitment efforts will follow FH recruitment SOPs for research. Florida Hospital (FH) Employees will not be individually targeted nor excluded from study participation based on employment. FH employees who engage the TRI-MD asking to participate in the study will be processed per standard consent procedures for participants. In addition, during the consent process, the study staff will review standard consent language stating that an employee's participation or lack of participation in the study will not affect their employment status or relationship with Florida Hospital.

Consent Process

We attest that all study staff delegated the authority to obtain informed consent will follow FH ORA SOP 08 (Informed Consent) and the INVESTIGATOR GUIDANCE: Informed Consent (HRP-802), as well as INVESTIGATOR GUIDANCE: Documentation of Informed Consent (HRP-803)".

Non-English Speaking Subjects

We will not recruit non-English speaking subjects.

Subjects who are not yet adults (infants, children, teenagers)

Not applicable

Cognitively Impaired Adults

Not applicable

Adults Unable to Consent

Not applicable

Documentation of Informed Consent Process

Documentation of the informed consent process is required to establish that the participant was accurately and adequately informed and that no study-related procedures were initiated prior to obtaining informed consent. A research team member will note in the source documentation the consent process, date consent was obtained and that consent was obtained prior to initiating any research procedures.

Waiver of Written Documentation of Consent or Waiver of Consent

Not applicable

Randomization

Not applicable

Study Visits

Please refer to **Appendix A** for corresponding Schedule of Activities.

Visit 1- Screening Visit/ Day -28 to -1 (outpatient, 2-3 hours): Subjects will take part in a screening visit in the TRI-MD clinic or the TRI-MD Diabetes Institute (DI) clinic.

After the participant has read and signed the study informed consent and agreed to study participation, the following procedures will be completed:

- Assessment of medical history; completion of Demographics and Health History Form **(Appendix B**)
- Physical exam
- Height and weight measurement
- BMI calculation
- Measurement of Waist and Hip Circumference
- Vital signs (Heart rate x 2, blood pressure x 2, respirations, temperature)
- AE/Conmed Assessment
- EKG
- Screening blood: CBC, CMP, HbA1c, thyroid function tests, lipid panel
- Urinalysis
- Urine drug screen
- Urine pregnancy test for female subjects of childbearing potential (all women are considered to be of childbearing potential unless they have undergone a hysterectomy, or have had an absence of menses for > 2 years)

Recruited candidates meeting all enrollment criteria will be scheduled for visits 2-3 (Phase 1). A subset of participants will be scheduled for visits 4-8 (Phase 2).

Female participants will be scheduled to fall within their Follicular phase Day 0 -14 (after menses are completed) of their menstruation cycle during the inpatient stay(s).

Phase 1 (24 individuals with T1D with a range of body weights; 9 matched controls)

Visit 2/ Day 1 (outpatient, 1-2 hours): Participants will complete:

- AE/ Conmed Assessment
- Vital Signs
- Urine Pregnancy test
- DEXA
- CGM placement (Type 1 diabetes group only)
- Activity Monitor Distribution

Visit 2/Day 1-7 (phone call). These two questionnaires will be completed through phone interviews done by the UNC Nutrition Obesity Research Center (UNC NORC). There will be a total of two phone calls between days 1 and 7.

- 24-hour Dietary Recall
- Previous Day Physical Activity Recall

Visit 3/ Day 8-11 (inpatient): Participants will be admitted to the TRI Clinical Research Unit (CRU) for 4 days (3 nights) for a comprehensive metabolic assessment including:

Day 8: Subjects will be admitted to the CRU in the *evening* and receive a calculated weight maintaining meal.

- Check into CRU
- AE/Conmed Assessment
- Vital Signs
- Activity monitor collection
- Inpatient Standard Diet- dinner meal only and a possible snack
- Overnight stay

Day 9: Subjects will complete following:

- AE/Conmed Assessment
- Vital Signs
- Inpatient standard diet- controlled meal three times a day and a possible snack
- Fasting blood samples will be collected for archiving and lipid panel
- FibroScan®
- Whole-body Magnetic Resonance Imaging (MRI)
- Magnetic Resonance Imaging and Spectroscopy (MRI/S)
- Magnetic Resonance Elastography (MRE)
- CGM placement (initial insertion for non-diabetes group)
- Stool collection and banking
- Paper Questionnaires (**Appendices C-M)**
- Overnight stay

Day 10: Subjects will complete following:

- AE/Conmed Assessment
- Vital Sigs
- Inpatient Standard Diet- controlled meal three times a day plus a possible snack.
- Check into calorimeter
- Whole Room Calorimetry for 23 hours
- Polar chest strap and Polar watch for heart rate monitoring
- Activity Monitor Distribution
- 24-hour urine collection
- Overnight stay

Day 11: Subjects will complete following:

- AE/ Conmed Assessment
- Vital Signs
- Inpatient Standard Diet- breakfast only
- Check out of calorimeter
- CGM collection (for Phase 1 only participants)
- Remove Polar chest strap and watch
- Activity Monitor collection
- Check out of CRU

Phase 2 (10 overweight/obese participants with T1D)

Visit 3/Day 11: Initial instruction in low carbohydrate, calorie restricted diet

After exiting the calorimeter, subjects participating in Phase 2 will meet with the research registered dietitian (RD). Counseling sessions will occur weekly on day 11, 18 +/- 2 and 25 +/- 2 to review their dietary adherence and adjust recommendations as necessary to meet the diet prescription of a low carbohydrate, calorie restricted diet. Additional counseling can occur by phone during the scheduled twice-weekly phone calls. In-person sessions with the nutritionist and/or registered dietitian will include audio recording of the session. Recordings of the RD sessions will be utilized to modify and refine the low carbohydrate education materials in preparation for the study's later dietary interventions, which will happen at UNC and Stanford. Training in ketone and glucose testing for safety.

Phone Calls (Phase 2 only)

Participants will be contacted by phone from a TRI- staff member approximately twice a week to monitor glycemic control, adjust insulin as necessary, and answer any questions they may have.

Visit 4/ Day 18 ±2 (outpatient):

- AE/ Conmed Assessment
- Vital Signs
- Home blood glucose and ketone test
- Urine Pregnancy test
- Diet counseling- low carbohydrate diet

Visit 4/Day 18 +/-2 through Day 24 (phone calls).

- At least 1 call to monitor diet adherence and tolerability
- At least 2 phone calls to monitor glycemic control, adjust insulin as necessary, and answer any questions they may have.
- Home glucose and ketone testing to continue.
- Continue outpatient low carbohydrate diet (self-selected foods based on counseling)

Visit 4-7/Day 18+/-2 through Day 35 (phone calls). These two questionnaires will be completed through phone interviews done by the UNC Nutrition Obesity Research Center (UNC NORC). There will be a total of two phone calls between days 18 and 35.

- 24-hour Dietary Recall

- Previous Day Physical Activity Recall

Visit 5/ Day 25±2 (outpatient):
- AE/ Conmed Assessment
- Vital Signs
- Home blood glucose and ketone test
- Urine Pregnancy test
- Diet counseling- low carbohydrate diet

Visit 5/Day 25 +/-2 through Day 31 (phone calls).
- At least 1 call to monitor diet adherence and tolerability
- At least 2 phone calls to monitor glycemic control, adjust insulin as necessary, and answer any questions they may have.
- Daily home glucose and ketone testing to continue.
- Continue outpatient low carbohydrate diet (self-selected foods based on counseling)

Visit 6/ Day 32 (outpatient):
- AE/Conmed Assessment
- Vital Signs
- Home blood glucose and ketone test
- Activity Monitor Distribution
- Pick up 3-day supply of controlled diet food (low carbohydrate, 500 calorie deficit)
- Consume outpatient low carbohydrate diet for 3 days (provided foods)

Visit 7/Day 35 (outpatient)
- AE/ Conmed Assessment
- Vital Signs
- Urine pregnancy test
- Home blood glucose and ketone test
- Pick up 3-day supply of controlled diet food (low carbohydrate, 500 calorie deficit)
- Consume outpatient low carbohydrate diet (provided foods)

Visit 8/ Day 37-40 (inpatient): Participants will be admitted to the TRI Clinical Research Unit (CRU) for 4 days (3 nights) for a comprehensive metabolic assessment including:

Day 37: Subjects will be admitted to the CRU in the *evening* and receive a calculated. low carbohydrate, 500 calorie deficit meal.
- Check into CRU
- AE/Conmed Assessment
- Vital signs
- Activity monitor collection
- Inpatient low carbohydrate, 500 calorie deficit diet- dinner meal only and possible snack
- Overnight stay

- Urine pregnancy
- Home glucose and ketone testing to continue

Day 38: Subjects will complete following:

- AE/Conmed Assessment
- Vital signs
- Inpatient low carbohydrate, 500 calorie deficit diet- controlled meal three times a day plus a possible snack .
- Fasting blood samples will be collected for archiving and Lipid panel
- MRI/S
- MRE
- Stool collection and banking
- Paper Questionnaires (**Appendices C-N)**
- Overnight stay
- Home glucose and ketone testing to continue

Day 39: Subjects will complete following:

- AE/Conmed Assessment
- Vital signs
- Inpatient low carbohydrate, 500 calorie deficit diet- controlled meal three times a day and possible snack.
- Check into calorimeter
- Whole Room Calorimetry for 23 hours
- Polar Chest strap and Polar watch for heart rate monitoring
- Activity Monitor Distribution
- 24-hour urine collection
- Overnight stay
- Home glucose and ketone testing to continue

Day 40: Subjects will complete following:

- AE/ Conmed Assessment
- Vital signs
- Inpatient low carbohydrate, 500 calorie deficit diet - breakfast only
- Check out of calorimeter
- Remove Polar chest strap and watch
- Activity Monitor collection
- Home glucose and ketone testing to continue
- CGM collection
- Check out of CRU

Analytical / Clinical procedures

Medical History and Physical Exam

A comprehensive health history will be obtained utilizing a standard Demographics and Health History Form that will be implemented for all aims of the grant (See **Appendix B**). A standard physical examination will be performed by a study physician, physician assistant, or nurse practitioner.

Anthropometric Screening

Body weight (calibrated scale), height, waist and hip circumference will be obtained while in a gown, without shoes. BMI will be calculated.

Screening Labs

The laboratory assessments below (at minimum) will be used to confirm study eligibility (Inclusion/Exclusion Criteria), as well as overall health status:

- complete blood count (CBC)
- comprehensive metabolic panel (CMP)
- HbA1C
- lipid panel
- thyroid function tests: thyroid stimulating hormone (TSH)
- urinalysis
- urine drug test
- urine pregnancy test, for female subjects of childbearing potential

Vital Sign Measurements

Measurement of vital signs will include heart rate (HR), blood pressure (BP), respirations and temperature will be measured sitting for at least 5 min.

Continuous Glucose Monitoring (CGM)

Starting at Visit 2, participants with type 1 diabetes will wear an unblinded continuous glucose monitor (CGM) for the duration of the study as a safety measure for glucose levels. Non-diabetes participants will have the CGM inserted during Visit 3 for the inpatient stay, for a data comparison. The CGM can be worn for up to 7 days at a time and will be replaced as needed. The CGM will not be worn in the MRI as the interference from CGM devices on MRI data has not been tested. It will be removed up to several days prior to the MRI and replaced after the MRI is completed. During time periods where blood glucose data is not available for CGM, point of care testing will continue.

The continuous glucose monitor will be placed by a member of the study team and participants will be instructed on the use and removal of the device, as well as reinsertion of the device as needed. During CGM wear, participants will be instructed to perform blood glucose

measurements approximately every 12 hours to calibrate the device, which can be completed by the participant or a trained clinical staff member. The FDA-approved system includes a sensor, transmitter, and receiver. It measures interstitial fluid glucose levels in the range of 40 mg/dl to 400 mg/dl every 5 minutes for up to 7 days.

Blood Glucose and Ketone testing

A small portable dual-purpose blood glucose and ketone meter, including lancets, test strips, and control solutions will be provided to the participant. A trained staff member will demonstrate and instruct the participant on proper techniques to check Blood Glucose and Ketone levels. The testing will be done via a small blood sample from a finger stick.

Measurement of 24-hour Energy Expenditure and Substrate Oxidation Rates: Whole Room Calorimeter

Whole room indirect calorimetry allows for the simultaneous measure of total energy expenditure (kcals/min) and substrate oxidation (RQ) in a free-living environment with multiple activities. Room calorimeters provide greater precision in the measure of oxygen consumption and carbon dioxide production as they provide a controlled testing environment and use more robust gas analyzers. Fat oxidation will be highest during periods of low activity [and fasting] as the metabolic system switches to relying on fat stores as the main energy source. Fat oxidation decreases as metabolic rate (kcal/min) increases through muscle activity.

The calorimeter will be maintained at thermoneutrality 75°F/22.5°C and a relative humidity of 40-%. The chamber is ventilated with medical air at a rate controlled by the chamber software to maintain CO_2 levels ~0.4% with a range of 20-90L/min. The room has two windows, and is furnished with a bed, desk and chair, television, sink and toilet with privacy curtain, a treadmill, a chilled air-locking pass-through for the storage of urine and fecal samples, and an air-locking food pass-through for meal delivery. Video cameras and microwave motion detectors continuously monitor the participant's movement.

Energy expenditure and substrate oxidations (including RQ) are calculated from O_2 consumption, CO_2 production and 24-hour urinary nitrogen excretion by using the equations established by Weir.

Brief overview of calorimetry routine: The participant will void prior to entry into the chamber. At this point, a timed urine collection will begin for the duration of the stay in the chamber. Participants will follow a set routine in the calorimeter that matches the activity routine in the CRU acclimatization period. Rest, meals, treadmill walking, desk time, and other activities are scripted to the minute and observed by the staff of the CRU and the Energy Expenditure Core via camera. Conditions during the stay will be standardized based on TRI's standard procedures (NCT 01967563). Inflow and chamber concentrations of O_2 and CO_2 are measured continuously, as are activity and environmental conditions and the rate of air flowing in to and out of the calorimeter via high accuracy mass flow controllers (MFCs). Urinary Nitrogen are measured over 24 hours for all days in the calorimeter. These values are fed into a computer system (PiLR™) that stores the data, calculates VO_2, VCO_2, and VCH_4, and converts these into EE

(kcal/minute) and RQ output every minute. Combined, fat, carbohydrate, and protein oxidations will be calculated as per Elia and Livesey and our recent publication in the American Journal of Clinical Nutrition.

During Phase 1, participants will consume a diet to achieve energy balance. During Phase 2, participants will consume a diet that reflects a 500-calorie deficit. Details of the calorimeter routine and energy balance procedures will be documented in a study specific procedure.

Continuous Heart Rate Monitoring

A chest strap (which is a Heart rate sensor) and Polar Watch will be worn by the participant during their 23-hour calorimetry chamber stay to continuously monitor the participant's heart rate while in the calorimetry room. The polar watch will record heart rate, time worn, steps, and energy expenditure (based on the entered age (in years only), weight, height, and gender). The collected data is considered exploratory and therefore does not pose a risk to the participant. The collected data will be uploaded to the software that accompanies the heart rate monitoring devices (Polarflow).

Dual Energy X-Ray Absorptiometry (DEXA)

DEXA Scans will be performed to measure body fat and estimate muscle mass using a GE Lunar iDXA whole-body scanner. The participant will remove all metal accessories, and may be asked to change into a hospital gown. The participant will lie on the DEXA table while the scanner arm emits low energy X-rays as it passes along the body. The scan takes up to 15 minutes and the radiation dose is less than 1 mrem, less than half the average daily radiation dose in America. A urine pregnancy test will be completed on all women of childbearing potential (all women except those with prior hysterectomy, tubal ligation, or absence of menses for $\geq$2 years) prior to the DEXA scan for safety.

FibroScan®

Approximation of liver fat and stiffness by FibroScan® will occur at scheduled visits outlined in Appendix A: Schedule of Events. FibroScan® uses ultrasound based VCTE™ (vibration-controlled transient elastography) to obtain Liver Stiffness Measurement (LSM) and Controlled Attenuation Parameter (CAP™); LSM is correlated to liver fibrosis stage, and CAP is correlated to hepatic steatosis. By using an ultrasound transducer probe, a painless, mechanical impulse is delivered to the skin above the liver using a low frequency elastic wave (50 MHz). The wave produces a mechanical deformation that moves towards and then through the liver. The velocity of the shear wave in the liver is measured and is directly proportional to its stiffness (stiffer tissue, faster velocity). Using a proprietary algorithm, the device determines whether the shear wave propagated into the liver and is valid. The LSM, expressed in kilopascals (kPa), is only calculated on valid measures. Liver stiffness measurement values range from 1.5 to 75 kPa; lower values indicate a more elastic liver. CAP™ measures the ultrasonic attenuation coefficient in the forward and return path through the liver at 3.5 MHz and is expressed in decibels/meter. CAP™ is evaluated simultaneously with LSM using the same radio-frequency data and is only appraised if the LSM is "valid." The specific FibroScan® model being utilized is the 502 Touch (Echosens, Paris, France).

Participants will lie on their back (supine) with their right arm raised in abduction to increase the intercostal space. A water-based gel will be applied to the skin and the transducer probe will then be placed on the participant's skin, in the intercostal space directly above the liver with slight pressure. At least ten valid measurements will be taken and the results will be immediate. To minimize the potential for confounding factors to affect the measurements, FibroScan® will be conducted following a fast (except water) of at least 4 hours and as much as practically possible. Only study staff members who are trained in the use of FibroScan® are permitted to acquire measures of liver stiffness and CAP™.

The test takes a total of about 10 minutes, with total scan time (including setup) of approximately 30 minutes.

Magnetic Resonance Imaging, Elastography and Spectroscopy

The goal of this test is to assess liver lipid content, liver stiffness, volumetric fat and organ volume quantitation using an Achieva 3T (Philips, Amsterdam, the Netherlands).

MRI/S: Intrahepatic Lipid (IHL) content will be measured using both imaging and spectroscopy. Scans will be performed under standardized conditions with participants in a supine position. Standard clinical MR Imaging, including 3-plane localization and T1 weighted images, will be completed to obtain anatomical images for voxel / slice localization and liver volume (to ascertain changes in hepatomegaly as compared to liver volume from whole body MRI). A 1H quadrature body coil (QBC) and torso XL coil will be used to measure intrahepatic fat stores. A single voxel point resolved spectroscopy (PRESS) sequence will be acquired from a 2 x 2 x 2 cm3 volume in the upper right lobe of the liver in an area that is free from heavy vascularization as determined from the scout images. Proton Density fat fraction (PDFF) imaging sequences will be performed covering at least the entirety of the liver. (Day 9 (Phase 1) and Day 38 (Phase 2)).

Whole-Body MRI: Volumetric measurement of fat, muscle, organs and bone will be completed across the whole body. These measurements are important for understanding metabolism since increases in size of metabolically active organs and adipose tissue distribution may contribute to alterations in energy expenditure. Scans will be performed under standardized conditions with participants in a supine position using the quadrature body coil and torso XL. Low resolution scans will be completed to determine appropriate positioning for high resolution images. (Day 9 only)

MRE: Liver stiffness will be measured using Magnetic Resonance Elastography (MRE). The MRE driver will be placed on the participant's abdomen for the duration of the MR acquisition. After acquisition of low resolution scans to determine positioning for MRE scans, the driver creates known pressure waves, which are applied during acquisition of tagged MR sequences, from which the liver stiffness is calculated. These sequences will be acquired over several sections of the liver. (Day 9 and Day 38).

Images which are degraded due to motion may be repeated as time permits. Individual scans and acquisition parameters are listed in the study specific imaging documents.

Total MR scan time will be approximately 90 minutes.

Resultant images will be analyzed using Analyze 11.0 (Biomedical Imaging Resource, Mayo Clinic, Rochester MN) to segment depots of fat (e.g. subcutaneous vs. visceral) as well as bone and muscle volume. Spectroscopy will be analyzed using jMRUI (the MRUI Consortium).

If required any Imaging (MRI, Fibroscan, and DEXA) can be scheduled +/- 2-day window. However, the preference would be to get the Imaging completed on the listed day (Day 1, 9 and/or Day 38 respectively).

Physical activity monitoring

Physical activity will be quantified with activity monitors using a small, portable accelerometer that can be worn on wrist and/or waist. Overall physical activity levels, daily changes, amount of time spent in sedentary, moderate, vigorous intensity categories and activity-associated energy expenditures will be extracted.

The participant will wear the monitor on the waist from Day 1 to Day 7 (it will be removed upon check-in in day 8) and day 32-36 (it will be removed upon check in on day 37). The goal is to collect at least 4 days of data to assess habitual activity. During Day 10-11 and Day 39-40, the participant will wear the monitor on the wrist and waist while in the calorimetry chamber. The goal of this dual assessment is to generate higher quality data for estimating activity energy expenditure that can then be translated to a free-living condition with high reproducibility. In addition, the wrist location will permit us to quantify sleeping quality. Poor sleep quality is a variable classically associated with negative health outcomes in patients with diabetes.

24-hour urine collection

During visit 3 and 8, all urine will be collected for measurement of total nitrogen, creatinine and glucose. Urine aliquots will also be archived for future use.

Stool Collection

A stool sample will be collected from participants during each of their inpatient stays. Participants will be provided with a container that fits over the commode and will be instructed on the proper way to provide the sample. Details of the stool collection kit and methods will be in an SSP and the Laboratory Manual.

In-patient diet procedures.

Participants will be fed meals in the TRI-MD dining room, clinical research unit or calorimeter. Participants will be provided a calculated weight-maintaining diet of 50% of calories as carbohydrate, 35% as fat, and 15% as protein (Phase 1) or a 500-kcal deficit diet with 14% of calories as carbohydrate, 58% as fat and 28% as protein (Phase 2). Given that quantifying energy balance is a primary goal of the study all meals will be observed by TRI staff. Participants will

be asked to consume 100% of meals. In the event this does not happen, food not consumed will be returned to the kitchen, weighed and recorded. In the calorimeter, macronutrient comparable units will be provided or removed to achieve energy balance or 500-calorie deficit.

Out-patient diet procedures.

Participants in Phase 2 will pick up meals containing 14% of calories from carbohydrate and a 500 kcal deficit on two occasions prior to the second inpatient evaluation. See Appendix A for details.

Dietary Counseling

The purpose of the low carbohydrate diet counseling will be to assist young adult, overweight study participants with Type 1 diabetes in adhering to a lower calorie diet (500 calorie deficit) that contains around 14% carbohydrate, 28% protein and 58% fat (majority polyunsaturated and monounsaturated fats). Study participants will attend an initial counseling session (60- 90 minutes) from a Registered Dietitian (RD) that covers general steps for problem solving, contents of eating a general healthy diet. An introduction to using MyFitnessPal to track their intake is done at Visit 2 (Day 1) and will be utilized thru Phase 1 (optional) and Phase 2 to help participant and study team to summarize food intake. The RD will also use the participant's 24-hour recall to discuss areas where lower carbohydrate and lower calorie foods could be substituted for foods the participant eats on a regular basis. Calorie balance will also be discussed as well as recommendations for insulin bolusing as carbohydrate intake is reduced and protein and fat intake is increased. Participants will also be provided with several sample low carbohydrate, low calorie meal plans, snack ideas, and food lists. At the end of the session, participants will work with the interventionist to set reasonable goals to adhere to the new low carbohydrate, low calorie diet for the next three weeks. Sessions 2 and 3 (30 minutes) will consist of the interventionist check-in and a more in-depth discussion of low carbohydrate and low-calorie consumption, as well as a concentration on saturated verses unsaturated fats.

24-hour Dietary Recall and Previous Day Physical Activity Recall (PDPAR)

These questionnaires will both be conducted by phone and are expected to take approximately 1 hour to complete.

As part of the Metabolic Chamber study, all participants will be asked to complete two 24-hour recall interviews. The 24-hour recall is a diet assessment instrument used to enable researchers to collect diet data on foods consumed by the participant during the previous 24 hours. A subset of participants of 10 participants will be asked to complete an additional two 24-hour recalls after being instructed on and living on a low carb diet.

To optimize the accuracy of the assessment of dietary intake data, 24-hour dietary recalls will be completed using the USDA multi-pass method and will be administered by trained diet recall technicians. Participants will self-report on intake of the previous 24 hours of food and beverage intake. Diet recalls will be conducted in English and will last 20-30 minutes. Dietary data will be entered and analyzed using NDS-R software (Nutrient Data System for Research, St. Paul, MN). Study participants will also be asked to complete the Previous Day Activity Recall after the 24-hr Dietary Recall. This portion of the interview will collect information about physical activity from

the previous 24-hour period and will take an additional 5-10 minutes to complete after the 24-hour recall. To increase precision of short-term usual dietary intake and physical activity, interviews will be completed on two non-consecutive days (one weekday and one weekend day).

Telephone recalls will be completed by the UNC Diet and Physical Activity Core (DPAC) in the NIH/NIDDK funded UNC Nutrition Obesity Research Center. All DPAC research staff are trained to collect 24-hour recalls using the USDA multiple pass method and are required to complete the Collaborative Institutional Training Initiative (CITI) Program for Social and Behavioral Research.

Study site staff will provide UNC with participant contact information including name, contact phone number, as well as best times to contact in order to conduct the diet/physical activity recalls via telephone. Participants will also be asked if they prefer to text or receive a phone call to arrange a time to conduct the 24-hour recall. Participants may opt out of texting and UNC DPAC will not contact via text.

All participant information provided to UNC, such as name, phone number, and best times to contact, will be stored in the Sheps Integrated Research System (SIRS), a secure online database developed and maintained by the UNC Cecil Sheps Center for Health Services Research. The participant information is imported into the Sheps secure database by the study site. Access to the participant information is only accessible via Sheps and login is only available to approved study site staff and trained UNC interviewers. All system login procedures and data submissions will be encrypted via the Secure Sockets Layer (SSL). Sheps Center programmers and research staff who work with sensitive data are required to complete appropriate HIPAA training with periodic updates, complete Sheps Center internal training, comply with the UNC IT Security Policies, and agree to the provisions of the Sheps Center's Rules of Behavior and Sanction Policy. The Sheps Center strives to implement reasonable security controls guided by FISMA, HIPAA, and OMB Circular A-130.

All data entered in the NDSR data analysis system as well as data coded for the PDPAR is deidentified. Participant IDs as provided to the UNC DPAC by the study site are used in lieu of any identifying information to enter data. There is no link from participant ID to sensitive participant information. Once all data collection is complete, all participant and related study information is permanently deleted from the UNC Sheps database.

Questionnaires During Inpatient Stay:

These questionnaires will be administered while participants are domiciled at TRI during both phases. They are paper questionnaires (**See Appendices C-N** for the content of each questionnaire) and are expected to take approximately 1-2 hours to complete.

Questionnaire	Description
Food Frequency	Habitual dietary patterns will be assessed using the 26-item Dietary Screener Questionnaire developed for the National Health and Nutrition Examination Survey.

Diabetes Eating Problem Survey	Diabetes related eating problems will be assessed using the Diabetes Eating Problem Survey (DEPS-R).
Dutch Eating Behavior Questionnaire	The Dutch Eating Behavior Questionnaire assesses emotional eating, externality, and restrained eating behavior.
BIS-15	The Barratt Impulsiveness Scale (BIS-15) assesses trait impulsivity, including non-planning, motor impulsivity, and attention impulsivity.
Food Craving Inventory	The Food Craving Inventory assess the degree of craving for a variety of foods.
Nutrition Knowledge Questionnaire	The Nutrition Knowledge Survey assesses healthful eating knowledge, carbohydrate counting, blood glucose response to foods and nutrition label reading. It has been validated in people with T1D.
Diabetes-Related Quality of Life	Diabetes-specific quality of life will be measured using the DCCT-validated measurement form.
Hypoglycemia Fear Survey (HFS)	The HFS will be utilized to assess anxieties and behaviors related to hypoglycemia.
Recent Physical Activity Questionnaire (RPAQ)	Self-reported physical activity patterns over the preceding month will be conducted using the Recent Physical Activity Questionnaire (RPAQ).
Sleep Disorders Questionnaire	The Sleep Disorders Questionnaire will be used to obtain sleep duration and quality over the preceding month.
CES-D	The Center for Epidemiological Studies-Depression (CES-D) is a 20-item measures that asks participants to rate how often over the past week they experienced symptoms associated with depression, such as restless sleep, poor appetite and feeling lonely.
Acceptability of Defined Diets (Only During Phase 2)	The diet acceptability form will be utilized following the low carbohydrate pilot to assess acceptability of the diet. An appropriate instrument with known psychometric properties to systematically assess acceptability of specific diets could not be identified in the literature. Therefore, we developed and are now piloting this simple assessment for this purpose (5 simple questions plus open-ended query).

Study Duration

The study will initiate participant recruitment in January 2018. The estimated duration to enroll all study participants is 6 months. The study consists of 3 visits (Phase 1) or 8 visits (Phase 2 participants only) including screening. Participation in Phase 1 will last 28-42 days (inclusive of the screening window) while it will be approximately 68 days for participants in Phase 2. We estimate that the study, including analysis will be completed in 12 months.

Materials of Human Origin: Collection, Preparation, Handling and Shipping

All biological materials will be obtained per the procedures described in study visits. For all study procedures, Study Specific Procedures (SSPs) have been prepared. Biospecimens will be collected and analyzed according to the SSPs and techniques established at the TRI-MD laboratory or at SBP laboratories.

Biospecimens collected for study-related endpoints (blood, urine and fecal samples), will be collected by a study team member, processed according to their respective protocols and stored at

TRI-MD until analysis takes place. Biospecimen samples will be stored in ultralow temperature freezers and liquid nitrogen dewars or other storage units located at the TRI-MD Laboratory Room 2404. The TRI-MD facility is secured via key card and equipped with a back-up generator system. Laboratory personnel in the facility have 24/7 key controlled access to the laboratory. Chain of custody of biospecimen samples is maintained through requisition forms and in the StarLIMS database. Specimen tubes are coded, and specimen requests and distribution are documented.

The biospecimens collected for this study will be separated into biospecimens that will be used for the study and biospecimens that were collected to be archived for future use. After study aims have been achieved and study related endpoints have been measured and analyzed, any remaining biospecimens will be stored at the TRI-MD Laboratory Room 2404 and will also be considered as "archived biospecimens."

Archived biospecimens will be used for any additional hypothesis-related experimentation or testing for the purposes of this study, consistent with the original aims, which cannot be predicted at the time the protocol is being developed due to the evolving nature of scientific exploration.

Archived biospecimens may be stored indefinitely for future research. Archived biospecimens could be used for separate research by both Florida Hospital scientists and scientists outside of Florida Hospital. This would be allowed if the research is consistent with the original aims of this study and if they have scientific merit as determined by the Principal Investigator, or with an additional review by the respective Program Director. For research outside of Florida Hospital, a Material Transfer Agreement will be obtained, which will govern the transfer and chain of custody of the biospecimens outside of FH.

Study Outcome Measures (Endpoints)

The following data variables and materials will be collected for analysis in this study:

- Patient information: age, gender, medical history.
- Physical exam: height, weight, calculation of BMI, heart rate, blood pressure, respirations, temperature.
- Blood samples: Fasting blood sample for hormonal analysis. A separate blood sample will be used for DNA sequencing and single-nucleotide polymorphism identification of genes affecting taste and metabolism.
- Stool samples: stool sample collection will be used for the measurement of phylogenetic (16S rDNA) and transcriptional (RNA-seq) analyses of fecal microbiota, metabolomics analyses including short-chain fatty acids.
- Body composition measures (DEXA)
- Body composition measures (MRI)
- 24-hour energy expenditure and substrate oxidation rates (calorimetry)
- Dietary questionnaires
- Diabetes questionnaires
- Diet adherence, glycemic control and insulin dosing (Phase 2)
- Liver fat percent via MRI/S
- Controlled Attenuation Parameters (marker of liver fat) via FibroScan®
- Liver stiffness via FibroScan® and MRE

- Organ size and body composition via MRI [including, but not limited to, brain, heart, kidneys, adipose tissue (visceral, subcutaneous, intramuscular), skeletal muscle]

Data Management and Quality Plan

Data De-identification

Participants will be enrolled using Cerner's Patient Protocol Manager; the application assigns each participant a unique participant identifier, or "PID". This PID is a code consisting of a combination of numerals and letters, which serves as the identifier for this participant for this research study and links them back to their hospital medical record and their protected health information (PHI). Access to the "link" between the PIDs, the PHI, and to the clinical data are only granted to the clinical research team as assigned on the Delegation of Authority Log. All the clinical research data is recorded in a de-identified fashion onto our paper source documents, which is then transcribed into our electronic case report forms, (CRF). The CRF is used for storage (a database) and facilitates analysis. Clinical data generated by research devices also uses the PID, and once the data has been transformed into interpretable results it is stored into the clinical research database, iDiscover. Both storage locations are secured and only assessable to the assigned clinical research team. The "link" will not be used to re-identify participants except in the event of a serious adverse event (SAE) requiring "unblinding" to treat the participant. The "link" will be stored in the Patient Protocol Manager and in i-Discover, where only the TRI-MD research team has access. These secure databases are stored/accessed on the Florida Hospital password-protected computer network. No one outside of Florida Hospital investigators or researchers will have access to the databases.

Data Confidentiality, Storage, and Retention

The identity and personal health information will be kept confidential to the extent permitted by the applicable laws and/or regulations and will not be made publicly available. If results of this study are published or presented, the identities will not be revealed. Confidentiality will be maintained during and after the study. This information is also included in the informed consent, which is discussed with participant prior to enrollment.

All paper sourced study documentation will be stored in the medical records room at TRI-MD, which is behind badge access doors, continuously locked, monitored and provides for limited access. The data records may also be stored as electronic records. This data is safeguarded so that only those on the research team have access to it via limited badge access to the medical records room (paper) or role relationship (electronic)). The electronic data is maintained under Adventist Information Technology security controls.

The duration of study data retention will be determined by governing FDA regulations and/or sponsor contract mandate (if applicable). TRI-MD retention policy is maintained in the Records Management Policy. Electronic de-identified data will be kept indefinitely in our data warehouse.

The Polar watch will record heart rate (from sensor in chest strap), time worn, steps and energy expenditure (based on the entered age, weight, height, and gender). There are no personal identifiers (ea. names or DOB) or PID's utilized during the recording. The collected data (mentioned above) will be immediately uploaded to the cloud based program provided by

Polarflow) which generated the report to be saved with the participant PID on the TRI -MD secure server.

Nutrition consult audio recording will be recorded on a portable recording device. After the session the data will be downloaded to the TRI H:drive as an audio file including the participants PID, initials and date of recording. Once confirmed that the data is correctly downloaded and saved the recording will be deleted from the recording device. The saved file will then be uploaded from the H:drive to the UNC Data system (RedCap) which is username and password protected.

Data Quality

Data quality and integrity will be governed by the system of ISO 9001 driven data management SOPs. These SOPs delineate how quality is built into the identification, collection, handling, and processing of the data.

The types of data collected may include data that are: (a) manually abstracted or electronically extracted from medical records, (b) observed in clinical exams, (c) obtained from laboratory and diagnostic tests, or from various biological monitoring devices, and (d) patient-reported items. Each data type can be understood by its source and the associated collection mechanism. Only the SOPs and associated workflows applicable to the study will be used.
Within the study quality control steps may include:

- Source Data Verification (SDV)
- Query Management
- Parallel Processing by a second party
- Completely re-processing a percentage of the data and comparing to the original
- Change Control; If changes are made they need to be documentation that covers what was changed, why it was changed, who changed it and when they changed.

Data Sharing (outside of Florida Hospital)

Deidentified data, potentially including archived biospecimens, will be shared with the University of North Carolina and Stanford University under a Data Use Agreement. This study is part of a Multi-PI NIH grant with three site PI's: Elizabeth Mayer-Davis, PhD (UNC Chapel Hill Site PI; UNC is the prime awardee and coordinating center), David Maahs, MD (Stanford Site PI) and Richard Pratley, MD, (TRI-MD Site PI). The Data Use Agreement(s) will identify the purpose for data sharing, the specific data elements to be shared, and will govern the sharing of data related to this study. Data will be de-identified, but a link/code is managed within an electronic research management system and maintained by a study coordinator.

Data sharing with other organizations would be allowed, as defined in respective Data Use Agreements, if the research is consistent with the original aims of this study and if they have scientific merit as determined by the three site Principal Investigators.

Some data sharing for the UNC to contact participants for the phone contact questionnaires (24-hour Dietary Recall and Previous Day Physical Activity Recall) requires the study site staff to provide UNC with participant contact information including name, contact phone number, as well as best times to contact in order to conduct the diet/physical activity recalls via telephone.

Participants will also be asked if they prefer to text or receive a phone call to arrange a time to conduct the 24-hour recall. Participants may opt out of texting and UNC DPAC will not contact via text (for more information see section 24-hour Dietary Recall and Previous Day Physical Activity Recall (PDPAR)).

Sample Size Determination

This is a pilot study from which we intend to collect information to perform larger studies. However, a total sample size of 33 will allow a power of 80% to detect a correlation of energy expenditure with glycemic control of 0.4 using a type 1 error of 0.1. Since this is a pilot study, this should be sufficient power for the stated modeling and estimation goals.

Statistical Analysis Plan

Overview of Statistical Plan: Similar to work we have done previously, calorimetry data will be utilized to determine the 24-hour energy cost of maintaining a specific level of glycemia and body weight, body composition and activity, using exponential modeling similar to that reported by Hall et al (2011). This will involve fitting a multivariate regression model, correcting for food intake, urinary glucose excretion, and energy expenditure while in the calorimeter, while also accounting for body composition, glycemia based on CGM while in the chamber, as well as HbA1c, age, sex, insulin dosing, and diabetes duration, and solving for the missing energy variable. Energy modeling using only variables available in the Phase 2 SMART pilot will also be derived and will be used to establish calorie prescriptions for the SMART pilot. For research questions related to RQ, substrate utilization, and metabolic flexibility, regression modeling will be done to determine differences between T1D and non- diabetic controls accounting for the matched design as well as age, gender, and other important co- variates.

Details of analytical procedures will be documented in a Statistical Analysis Plan.

Primary Objective Analysis

To develop a comprehensive model of energy balance and metabolic flexibility for T1D that accounts for the degree of hyperglycemia and differences in body composition we will combine data from calorimetry, body composition, glycemic control and demographics to generate a regression equation based on the variables that impact energy requirements. General linear models with multiple covariates (age, sex, lean body mass, fat mass, HbA1c, mean glucose levels on CGM will be employed initially. Additional mathematical modeling tools may also be implemented. This is the first study of its kind and is intended to generate pilot data for understanding energy balance across the spectrum of body weight in individuals with T1D. Therefore, several methods may need to be employed to generate the final model that will be used in latter phases of the overall grant.

Secondary Objective Analysis

This objective is piloting a low carbohydrate diet to generate the first comprehensive metabolic data set in individuals with T1D across a range of body weights. Here the goal is primarily to assess the tolerability and safety of a restricted carbohydrate, hypocaloric diet in patients with T1D and to evaluate the instruction methods and tools necessary to

implement such as diet. In addition, we will assess the effects of the diet n energy expenditure to further enhance the modeling performed in the primary objective.

Potential Risks and Benefits

Potential Benefits

Participation in this study will not result in any direct benefits to the subjects in this study, however participation in the screening may identify previously unrecognized medical conditions, which may provide a general health risk assessment. Subjects will also receive some general medical information, including laboratory testing and medical examination. No promise can be made concerning the study outcome, because results from a clinical research study cannot be predicted.

Potential Risks

Venipuncture: The placement of intravenous needles may cause transient pain, vasovagal syncope, and may also result in infection, bruising, bleeding, and/or clotting at the site of needle insertion. The application of direct pressure at the catheterization site will be used to help prevent these symptoms. There is a possibility that a catheter placement would be unsuccessful or need to be removed. If this should occur, another catheter would be placed. It is possible that this may occur more than once during the subject's participation in the protocol. Staff trained and certified in the SOP will be used.

Protection Against Risk:

- All venipuncture will be conducted by qualified staff using aseptic techniques to reduce these risks.
- We are recruiting young, healthy individuals and do not anticipate the blood volume for this study to be problematic. However, the following precautions will be taken.

Total amount of blood that will be drawn throughout the study will be approximately 65 ml.

DEXA scan: There is a very small risk of cancer with excessive exposure to any radiation. Each scan takes about 15 minutes and the radiation dose for one scan is approximately 0.6 mrem, which is equal to about half a day of background radiation. This radiation exposure is below the guideline of 5000 mrem per year allowed for research participants by the NIH Radiation Safety Committee. The use of the DEXA scan apparatus may cause some minimal discomfort in claustrophobic participants and may cause some minimal back pain in a small minority of the individuals.

Protection against Risk:

- The radiation dose from the scans is less than a chest x-ray, or about the same amount a person would receive from one day of background radiation from the sun.
- A urine pregnancy test will be done prior to scans of all women of childbearing potential (all women except those with prior hysterectomy, tubal ligation, or absence of menses for $\geq$2 years).

Magnetic Resonance Spectroscopy: There are no known biological risks associated with magnetic resonance spectroscopy. Some short-term discomfort may be experienced. The short-term risks associated with MRI are minimal, but include heating, loud noises and claustrophobia. There are some people who should not undergo MRI; the contraindication is largely based on the presence of certain metal objects within a person (i.e. pacemaker, aneurysm clip, metal fragments, etc.).

Protection against Risk:

- There will be a strict safety screening protocol, to ensure any people with contraindications are excluded from volunteering in MR portion of study
- Incidental Findings: There will be no diagnostic analysis associated with any of the MR sequences used in this protocol; the images will not be sent for radiology review. However, some of the MR images we obtain as part of this protocol may show incidental medical findings. In the case where a medical abnormality is apparent on an image, the image will first be reviewed by an investigator on this protocol. If the abnormality is confirmed, then the participant will be instructed to seek medical attention from their health care provider

Activity Monitoring: There are no risks associated with the wearing of activity monitors.

Heart Rate Monitoring: There are no risks associated with the wearing of the chest strap and Polar Watch.

Metabolic 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 participants' physical health. Claustrophobia is an exclusionary criterion. All participants will be given an opportunity to experience the metabolic chamber prior to enrollment in the study.

Protection against Risk:

- Participants will be closely monitored to ensure they are comfortable while in the calorimeter.

Low Carbohydrate Diet: Low carbohydrate diets could lead to hypoglycemia and diabetic ketoacidosis (DKA).

Protection against Risk:

Participants will have at least bi-weekly calls to assess maintenance of glycemic control. Furthermore, participants will be asked to check their blood sugar approximately every 12 hours plus blood ketones at least once per day. Participants will keep a log of their daily values and contact the study team immediately (see below) if values are out of range. If the ketone values are elevated (> 0.6 mmol/L) or if they display any symptoms of elevated ketone levels, they will contact the investigator immediately. Early symptoms of elevated Ketone levels include; polydipsia and polyuria several days before the onset of DKA. Other common symptoms include generalized weakness, weight loss, nausea, vomiting, and abdominal pain. Prolonged elevated levels of ketones can lead to swelling in brain, loss of consciousness, diabetic coma and death.
Contact:

Translational Research Institute for Metabolism and Diabetes 301 East Princeton Street, Orlando FL 32804
Phone Number: 407-303-7100.

Participants are also wearing a continuous glucose monitor for the duration of the of the study that will alert them to glucose trends and allow them to take corrective/preventive action as necessary.

Participants will be provided with Hypoglycemia, Hyperglycemia (visit 2) and diabetic ketoacidosis (visit 4) information/instructions sheets

Continuous Glucose Monitor Risks: The risks of wearing the CGM are minimal. Bruising, redness, discomfort, and some bleeding can occur. Mild skin irritation is common. Rarely an infection can occur at the site of CGM sensor needle placement. Any infection, or sign of infection, will be treated immediately. An allergic reaction to the tape used to hold the sensor in place is possible. If there is pain, redness, irritation, or rash at insertion site, the sensor will be removed and re-inserted in a different site.

Protection against Risk:

- CGM placement will be conducted by qualified staff using aseptic techniques to reduce these risks. Participants will be trained on proper insertion techniques so that they can replace sensors at home as necessary.

Other Risks: In addition to the risks listed above, participants may experience a previously unknown risk or side effect. They will be asked in advance about any previous injury, which could prevent them from participating in this test.

Mitigation of Risks

Invasive procedures (blood sampling and vessel cannulation) will be conducted at the TRI by qualified staff following institutional policies and procedures including sterile dressing to the site.

Provisions to Protect the Privacy Interest of Subjects

Subjects will be assigned unique identifiers for study-related records. All precautions will be taken to make sure that only authorized individuals will access subject research records. The collection of sensitive information about subjects will be limited to minimum necessary to achieve the aims of the research, so that no unneeded sensitive information will be collected.

Early Withdrawal of Subjects

Investigator Withdrawal of Subjects

The participation in this study may be stopped at any time by the study PI without the participant's consent because:

- The study Medical investigator thinks it necessary for subject's health or safety;
- Participant has not followed study instructions;
- Participant has not followed study supplement consumption compliance
- Participant has not followed dietary compliance
- The TRI-MD has stopped the study;
- Administrative reasons require the participant's withdrawal;

Subject Request for Withdrawal from Study

Participation in this study is voluntary. Participants may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. If a participant leaves the study before the final regularly scheduled visit, she/he may be asked by the study doctor to make a final visit for some 'end-of–study' procedures. This is to make sure that there are no safety concerns

Data Collection and Follow-up for Withdrawn Subjects

Participants who request withdrawal or who are withdrawn by the PI from the study will have their data maintained in the research database up to the point of withdrawal. This data will be included in subsequent analysis because a participant may have withdrawn due to possible drug side effects and keeping these participants in the analysis is essential for study validity.

Adverse Event Reporting

An adverse event (AE) is an untoward medical occurrence in a subject administered a product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, whether or not considered related to the product. Each participant is evaluated for adverse events at every study visit. Any event that is reported to the study staff and which meets the criteria of an adverse event will be documented as such and graded as to its attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol) and severity (mild, moderate, or severe). Any severe and/or unanticipated adverse event will be immediately reported to the IRB.

Recording and Notification of Adverse Events

At each contact with the subject, the study team will seek information on adverse events by specific questioning and, as appropriate by the Provider through examination. Information on all adverse events will be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results will be recorded in the source document.

All adverse and unexpected events will be reported according to Florida Hospital IRB guidelines.

Safety Monitoring Plan

We will provide the following safety and monitoring procedures, in compliance with the policy of the NIH that all investigative sites provide a plan regarding monitoring and oversight of the conduct of the clinical trial to ensure the safety of volunteers and the validity and integrity of the data. Data and safety monitoring will be performed in accordance with all local IRB, NIH, and other applicable federal regulatory guidelines. Research and treatment procedures will also be conducted in accordance with the Principles of Good Clinical Practice (GCP) guidelines. Standard FH and SBPMDI IRB procedures and infrastructure for data and safety monitoring will be utilized.

Safety Monitoring

All adverse events will be documented by the study team. The study coordinator will review adverse events with a medical provider (PA, ARNP, and/or MD) on a routine basis. In addition, the Translational Research Institute has a standing committee that meets monthly to review all adverse events in our clinical trials and will additionally be charged with review of the study. Adverse events will be reported to the Florida Hospital IRB as required per HRP 801-INVESTIGATOR GUIDANCE: Prompt Reporting Requirements.

Data and Safety Monitoring Board (DSMB) or Equivalent

Although a DSMB is not required for this study, we will report to the ACT1ON DSMB who will evaluate safety and data quality for the grant in its entirety. Drs. Mayer-Davis, Maahs and Pratley will represent the ACT1ON consortium in interactions with the DSMB. Their focus will be the statistical analyses related to Aim 3 of the grant which will be conducted at Stanford and UNC Chapel Hill. The DSMB will review the final study protocol and any significant changes to the protocol over the course of the study especially as related to participant burden or safety. The DSMB will monitor and advise on study participant accrual and retention, progress and completeness for all standardized measurement visits and attendance at intervention sessions. Adverse events will be monitored, as well as staff training and certifications. A summary of the DSMB report will be sent to both the local site IRBs and NIDDK as part of the annual progress report. The DSMB has already been established and consists of external experts in in endocrinology, nutrition, health psychology and behavior change, and study design and statistical analysis. The DSMB convened for its inaugural meeting on 9/28/2017. Please refer to grant documents for details on committee membership/

Ethical Considerations

Participation in this study is voluntary. Subjects may decide not to participate in this study or may withdraw from this study at any time without penalty or loss of benefits. No vulnerable populations will be studied in this protocol.

Sharing of Results with Subjects

Participants will be offered the opportunity to meet with the Principal Investigator or designated medical staff to review the results of their lab assessments or other standard clinical data. Copies of their testing results will be made available to the participants upon request. In addition, the Principal Investigator or designated study staff will provide an overview of the study's outcome to the participant if he or she requests the information.

Funding Source

Phase 1 is funded through a National Institutes of Health Grant awarded to the University of North Carolina with Florida Hospital as a Subcontract. Procedures not originally in the grant along with Phase 2 will be funded through internal funds.

Subject Stipends or Payments

After the participant’s completion of study visits, a Mastercard® payment will be processed for each study phase. Mastercard® payments may take up 3 business days to be processed, once requested. Enrolled participants that successfully complete the study requirements for Phase 1 will

receive a one-time payment of $660. If participant has successfully completed Phase 2 the total stipend is$ 1495 (Phase 1 and Phase 2 combined). Screened participants that that do not meet study criteria will receive a one-time payment of $50. Participants that voluntarily or administratively withdrawn due to lack of compliance will not receive any payment. Participants that are withdrawn due to: a 1) health or safety concerns (as assessed by the medical investigator), b) discontinuation of the study, or c) other administrative reasons (not related to compliance) will receive prorated payments dependent on time of participation (i.e. $25 for screening, $25 for each completed visit).

Participants who agree to take part in this study will be paid for completed study visits according to the following schedule:

Phase 1	
Visit 1 (Screening)	$ 50
Visit 2 (Day 1)	$ 75
Visit 3 (Day 8)	$ 125
Visit 3 (Day 9)	$ 125
Visit 3 (Day 10)	$ 200
Visit 3 (Day 11)	$ 85
Total Phase 1	**$ 660**

Phase 2	
Visit 4	$ 75
Visit 5	$ 75
Visit 6	$ 75
Visit 7	$ 75
Visit 8 (Day 37)	$ 125
Visit 8 (Day 38)	$ 125
Visit 8 (Day 39)	$ 200
Visit 8 (Day 40)	$ 85
Total Phase 2	**$ 835**

Publication Plan

TRI-MD will publicly disclose data. Whenever possible, data dissemination will occur through presentation at major scientific conferences and/or publication in peer-reviewed journals, and will be complete, accurate, balanced, and timely. We attest that the TRI faculty and staff will adhere to POLICY-TRI-ADM-005 (Access to Clinical Trial Data for Publication Purposes). Since this study is one component of a multi-center study, publications, abstracts and presentations will be reviewed and approved by a Publications and Presentation Committee as delineated in the submitted grant documents.

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Appendix A: Schedule of Activities

	Phase 1						Phase 2							
	Visit 1	**Visit 2**	**Visit 3**				**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**			
	SV Day -28 to Day -1	Day 1	Day 8	Day 9	Day 10	Day 11 + 28	Day 18 +/- 2	Day 25 +/-2	Day 32	Day 35	Day 37	Day 38	Day 39	Day 40
Informed Consent	X													
History and Physical	X													
Height	X													
Weight	X													
Calculated BMI	X													
Waist/Hip Circumference	X													
Vital Signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X
EKG	X													
AE/Conmed Assessment	X	X	X	X	X	X	X	X	X	X	X	X	X	X
CBC	X													
CMP	X													
HbA1c	X													
TSH	X													
Lipid Panel	X			X								X		
Urinalysis	X													
Glucose and Ketone test[1] (point of care)						X	X	X	X	X	X	X	X	X
Toxicology screen	X													
Urine pregnancy test	X	X					X	X	X	X	X			
DEXA		X												
24-hour Dietary Recall[2]		X					X							
Previous Day Physical Activity Recall (PDPAR)		X					X							
Bi-weekly calls						X	X	X	X	X				
CGM Placement/Collection (T1D group)[3]		X				X								X
CGM Placement/Collection (Healthy group)				X		X								
Activity Monitor Distribution		X							X					
Activity Monitor Collection			X								X			
Check into CRU			X								X			
Overnight Stay			X	X	X						X	X	X	
Check out of CRU						X								X
Inpatient Standard Diet			X	X	X	X								
Diet Counseling- Low carb, hypocaloric[4]						X	X	X						
Pick up outpatient controlled diet- Low carb, hypocaloric[4]									X	X				
Outpatient Low Carb Diet									X	X				
Inpatient low carb diet											X	X	X	X

	Phase 1						Phase 2							
	Visit 1	**Visit 2**	**Visit 3**				**Visit 4**	**Visit 5**	**Visit 6**	**Visit 7**	**Visit 8**			
	SV Day -28 to Day -1	Day 1	Day 8	Day 9	Day 10	Day 11 + 28	Day 18 +/- 2	Day 25 +/-2	Day 32	Day 35	Day 37	Day 38	Day 39	Day 40
Blood Collection for Archiving				X								X		
FibroScan				X										
MRE				X								X		
MRI/S				X								X		
Whole Body MR				X										
Stool Collection and Banking				X								X		
Diabetes, Ingestive Behavior, Nutrition Knowledge Questionnaires [5]				X								X		
Acceptability of Defined Diets Questionnaire												X		
Check into calorimeter					X								X	
Whole Room Calorimetry- 23 hours					X								X	
Polar Heart Rate Monitor placement					X								X	
Polar Heart Rate Monitor collection						X								X
24-hour urine collection					X								X	
Check out of calorimeter						X								X

1. Glucose testing to be performed at least twice per day and ketone testing at least once per day
2. Two phone calls will be conducted by UNC between Day 1 through Day 7 and Day 18 through Day 35.
3. Participant will continuously wear CGM until collection at either the end of Phase 1 or Phase 2 as appropriate. The site must be changed at least every 7 days.
4. Phase II participants only
5. Food Frequency Questionnaire, Diabetes Eating Problem Survey, Dutch Eating Behavior Questionnaire, Barratt Impulsiveness Scale (BIS-15), Food Craving Inventory, Nutrition Knowledge Survey, Diabetes Quality of Life, Hypoglycemia Fear Survey (HFS), Recent Physical Activity Questionnaire (RPAQ), Sleep Disorder Questionnaire, The Center for Epidemiological Studies-Depression (CES-D)