CLINICAL STUDY PROTOCOL
Interventional Drug or Biologic

Metabolic and Neurological Changes Induced by a Very Low Carbohydrate Diet in Youth with Type 1 Diabetes

Protocol Number
HIC 2000029479

Protocol Version
08/11/2023
13

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IND Number:
IND Holder:
NCT Number: NCT04955691
Study Phase (1, 2, 3, or 4-postmarketing):
Confidentiality Statement:

REVISION HISTORY:

Revision #	Version Date
1	7/26/2021
2	8/24/2021
3	9/29/2021
4	11/10/2021
5	1/5/2022
6	2/14/2022
7	3/9/2022
9	6/27/2022
10	7/11/2022
11	8/25/2022
12	04/25/2023
13	8/11/2023

Synopsis

Primary Objective The primary objective of this randomized crossover study is to determine whether a very low carbohydrate diet improves glycemic changes (measured by continuous glucose monitors) when compared to a standard carbohydrate diet in youth with type 1 diabetes.
Secondary Objective (if applicable) The secondary objectives of the study are to compare metabolic responses (epinephrine, glucagon, cortisol) to hypoglycemia when youth with type 1 diabetes follow a low carbohydrate versus standard carbohydrate diet using the 2-step hyperinsulinemic euglycemic-hypoglycemic clamp and stable isotope infusions. The euglycemic portion of the clamp will allow us to determine the hepatic and peripheral insulin sensitivity changes after 2 weeks on each diet.
Study Duration 4 years
Study Design This is a prospective randomized crossover study of youth with type 1 diabetes.
Number of Study Sites Yale New Haven Hospital, Hospital Research Unit (HRU), Church Street Research Unit (CSRU), Masonicare, and the Pediatric Diabetes Research Center at 1 Long Wharf Drive, Suite 503.
Study Population This study will enroll 20 participants with type 1 diabetes between the ages of 12 and <25 years of age from any racial or ethnic group. Participants will be recruited from the Pediatric and Adult Diabetes Clinics at Yale or those in the community capable of traveling to Yale for study procedures.
Number of Participants 20
Primary Outcome Variables **Aim 1:** Primary Efficacy Outcome: Percent time spent in the target range of 70-180 mg/dL during 2 weeks on each diet. Secondary Safety Outcomes: We will measure the percent time spent in the hypoglycemic (<54 mg/dL, <70 mg/dL) and hyperglycemic (>180 mg/dL, >250 mg/dL) ranges. We will also measure the average sensor glucose values, standard deviation, and coefficient of variation during each 2-week diet period. We will compare the proportion of days with elevated fasting beta-hydroxybutyrate levels (≥ 0.6 mmol/L) during each diet period. Number of episodes of diabetic ketoacidosis and severe hypoglycemic events during each period (baseline, diet periods, and washout period) will also be reported.
Secondary and Exploratory Outcome Variables (if applicable) **Aim 2:** Primary Safety Outcomes: Mean levels of glucagon produced in response to hypoglycemia after each diet will be measured using a 2-step hyperinsulinemic euglycemic-hypoglycemic clamp. These will be measured after 15, 30, 45, and 60 minutes of euglycemia and hypoglycemia. Secondary Safety Outcomes: Measurements of mean levels of other counterregulatory hormones (cortisol, epinephrine, norepinephrine), beta-hydroxybutyrate that are produced in response to hypoglycemia during the clamp will be compared after each diet period during the clamp procedures. Additionally. fasting levels of glucagon, free fatty acids, beta-hydroxybutyrate and IGF-1 (measure of growth hormone secretion) prior to the clamp will be compared after each diet period. Secondary Efficacy Outcomes: Hepatic glucose production and adipose tissue lipolysis will be measured using stable isotope infusions during the euglycemic and

hypoglycemic portions of the clamp. We will compare differences in hepatic glucose production during each clamp between a standard and very low carbohydrate diet. We will also measure glycogenolysis and gluconeogenesis using deuterated water.

Aim 3:

Primary Safety Outcome: We will measure hypoglycemia awareness symptoms after 20-30 minutes of euglycemia and hypoglycemia during each clamp using a validated Edinburgh Hypoglycemia Symptom questionnaire. Scores during euglycemia will be compared to scores during hypoglycemia for each clamp. Scores during hypoglycemia after each diet period will also be compared.

Primary Efficacy Outcome: To describe how participants feel about and react to following a very low carbohydrate diet, interviews will be conducted over zoom (HIPAA compliant). Data will be coded and emerging patterns will be combined into descriptive themes.

Abbreviations

Abbreviation	Explanation
T1D	**Type 1 diabetes**
VLCD	**Very low carbohydrate diet**
HRU	**Hospital Research Unit**
CSRU	**Church Street Research Unit**

Glossary of Terms

Glossary	Explanation

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1 Introduction

1.1 Introductory Statement: As more adolescents with type 1 diabetes (T1D) opt to follow very low carbohydrate diets to improve glucose control, it is becoming increasingly important to study the metabolic and neurological changes that occur on these diets and how these effect patient safety. Understanding the impact of dietary changes on metabolic and neurological factors will help guide clinical advice about the overall safety and efficacy of these diets in the pediatric T1D population. This work will be the basis of future studies testing dietary interventions to improve glycemic control and ensure that these dietary interventions are safe for growing youth with T1D.

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2 Background

2.1.1 Preclinical Experience

Data from the Diabetes Control and Complications Trial (DCCT) demonstrated that lowering the A1c by intensive insulin management to near-normal glycemic levels decreases the risk of complications related to diabetes (1). However, intensive management in the DCCT was accompanied by marked increases in severe hypoglycemia and more recent data have implicated increased insulin doses as risk factors for cardiovascular disease in T1D 7. One approach to achieve targeted glycemic control with less insulin exposure is through the use of a very low carbohydrate diet (VLCD), defined as limiting carbohydrate intake to 50 grams per day or less 8. In adults with T1D, these diets have been shown to achieve exceptional glycemic control with physiologically normal A1c values with lower total daily insulin doses due to decreased mealtime insulin for low amounts of carbohydrate intake. (9,10)

In this study, we plan to look at very low carbohydrate diets, composed of less than 15% of calories from carbohydrates, or about 75 grams of carbohydrates per day and compare them to standard carbohydrate diets composed of at least 40% of calories from carbohydrates, or about 200 grams of carbohydrates per day. In clinical practice, both of these levels of carbohydrate intake are feasible to maintain long-term, and thus were chosen for this study.

2.1.2 Clinical Experience

In an unpublished study (NCT04082884) of youth with T1D following a VLCD, 4 participants ages 14-20 showed improvement in continuous glucose monitor derived glucose metrics, increasing the time in the target range while decreasing hypoglycemia and hyperglycemia. No participants have had any severe hypoglycemic episodes or diabetic ketoacidosis. Ketone levels for youth in this study ranged from 0 to 0.7 mmol/mol

Table 1. Mean sensor glucose and time spent in each of the glycemic ranges comparing standard carbohydrate and high protein very low carbohydrate diets. Std Dev - standard deviation.

	V22		V23		V24		V25	
Type of diet	STD	VLCD	STD	VLCD	STD	VLCD	STD	VLCD
GMI (%)	6.8	6.6	8.4	7.8	7.8	7.0	8.7	8.4
Mean Glucose (mg/dL)	144	139	214	189	189	153	225	221
Std Dev (mg/dL)	66	52	92	85	92	70	80	84
<54 mg/dL	6	1	<1	1	2	1	<1	<1
<70 mg/dL	8	5	1	2	3	3	1	1
70-180 mg/dL	55	73	39	46	51	71	29	36
180-250 mg/dL	25	17	27	28	21	16	33	29
>250 mg/dL	6	4	32	23	23	9	36	33
Sensor Use	85%	76%	100%	79%	100%	86%	100%	100%

2.2 Background/prevalence of research topic

As recently reported by the Type 1 Diabetes Exchange (T1DX) Registry, adolescents with type 1 diabetes (T1D) was the age group that had the highest hemoglobin A1c (A1c) levels compared to older participants; young adults aged 10-25 years were a close second (1). While the American Diabetes Association (ADA) previously recommended youth target A1c of <7.5% and adults <7%, only 17% of youth and 21% of young adults in the T1DX achieved these targets despite increased penetrance of diabetes technologies into clinical care (2,3). More recently, the target A1c level was lowered for adolescents to 7% (4). These

data suggest a need for novel approaches to improve glycemic control in adolescents and young adults with T1D.

Even for youth who are engaged in actively managing T1D, postprandial hyperglycemia continues to pose a challenge for many reasons, including, delayed insulin absorption and action compared to absorption of glucose from the gastrointestinal tract and dysregulated glucagon secretion after mixed meals (5). Moreover, postprandial glycemic changes can differ substantially depending on the macronutrient composition of the meal. Specifically, meals with reduced carbohydrate but increased fat and protein content will lessen immediate postprandial hyperglycemia (6). For many, being able to avoid carbohydrates and adhere to a very low carbohydrate diet may be easier than administering varying amounts of insulin for variable amounts of carbohydrate intake.

Importantly, VLCDs have not been formally studied in adolescents and young adults with T1D. Observational data on children using a VLCD are also conflicting. A small case series of 6 children with T1D on a low carbohydrate diet showed deficits in anthropomorphic measurements and elevated lipids, though in some cases a caloric deficit was also reported(7). Conversely, a recent observational study of 131 children with T1D on a VLCD reported no significant change in height percentile or SDS after initiating the diet (8). Prior studies have explored use of low carbohydrate diets in T1D, but none have specifically monitored for ketosis. In those studies, it is likely that the lack of carbohydrate intake led to lower insulin needs, thus increasing lipolysis and ketogenesis, and levels of ketosis while on this type of diet (9). Thus, this study aims to evaluate the metabolic and neurological effects of hypoglycemia as they relate to the diet, which is a key concern from clinicians managing patients on this diet.

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3 Rationale/Significance

3.1 Problem Statement: Youth with type 1 diabetes struggle to attain healthy glucose targets despite advances in diabetes technology. Adjunctive therapies to optimize glycemic control have not shown any benefit for youth with type 1 diabetes (10). Observational data in adults with T1D suggest that a very low carbohydrate diets reduce both hyperglycemia and hypoglycemia (8). However, the efficacy (time in target range) and safety of short-term use of very low carbohydrate diets in adolescents with diabetes has not been established.

3.2 Purpose of Study/Potential Impact: Very low carbohydrate diets have not been prospectively studied in adolescents and young adults with T1D. The participants in this study are likely to experience improvement in their blood glucose management when following a very low carbohydrate diet. This study will help guide treatment decisions, safety considerations, and dietary recommendations for national and international diabetes associations. The studies carried out in this proposal will provide important safety and efficacy information about very low carbohydrate diets in adolescents with T1D and have the potential to change the current standards of care.

3.2.1 Potential Risks

- **Hypoglycemia/hyperglycemia (hyperinsulinemic euglycemic-hypoglycemic clamp)**: Hypoglycemia and hyperglycemia are risks of the hyperinsulinemic clamp procedures. During each clamp, IV insulin is infused which can cause hypoglycemia. To protect against unanticipated hypoglycemia, blood glucose levels are measured by a reliable device (YSI 2300) frequently throughout the study. Dextrose infusion will be adjusted through the IV to ensure the glucose remains at the target level. Hypoglycemia will occur during this study as this is a part of the clamp procedure. Our pediatric diabetes team is very experienced in conducting these studies; Dr. Nally will work with Dr. Sherr and Dr. Herzog to fine-tune her clamp skills. To prevent severe hypoglycemia, glucose levels will be monitored frequently and dextrose infusions can be adjusted immediately.
- **Stable Isotopes**: Non-radioactive stable isotope infusions will be performed during this study. Despite the theoretical risk of infection with an infusion of stable isotopes, our team has been using isotopes during clamp studies for over 17 years in children and adolescents and have not experienced any adverse events (Appendix 1).
- **Risk of indwelling catheter**: Intravenous catheters pose a potential risk of hematoma, thrombophlebitis, bleeding at the catheter insertion site, and rarely, infection.
- **Risk of anemia**: There is a risk of anemia in any study with frequent blood draws. We have calculated the amount of blood needed for the clamp procedures and will not exceed volume limits.
- **Risk of Indirect Calorimetry:** The air under the hood may become warm and stuffy, which some subjects find uncomfortable. Rarely, subjects may feel nauseated and/or claustrophobic while under the hood.
- **Risks of the Very Low Carbohydrate Diet***:* Previous studies have suggested that switching to a very low carbohydrate diet can cause symptoms including nausea, vomiting, abdominal pain, constipation, fatigue, and lightheadedness. These symptoms will be screened for at follow up visits throughout the study. Continuous Glucose Monitors will be worn by all participants during the study to alert participants of hypoglycemia and hyperglycemia. Participants will be instructed to reach out to research staff if they are having issues with hyperglycemia and hypoglycemia. At each visit, participants will receive guidance on glucose monitoring, food intake, and treatment of hypoglycemia.

- **Risks of ketosis**: Risks of the very low carbohydrate diet include the development of elevated ketone levels. These will be monitored frequently in the outpatient setting and all supplies will be provided to participants. Participants will also be educated on warning signs of DKA and will be instructed to call the study team for any elevated ketone levels > 1 mmol/L or if they have symptoms of ketosis (nausea, vomiting, headache). If a participant develops DKA, they will be withdrawn from the study. Hyperglycemic events will be classified as diabetic ketoacidosis (DKA) if all of the following are present:
    - Symptoms of polyuria, polydipsia, nausea, or vomiting;
    - Serum ketone level >1.5 mmol/L or large/moderate urine ketones;
    - Serum bicarbonate <15 or venous pH <7.24 or arterial pH <7.3;
    - Treatment provided in a healthcare facility;
- **Risk of loss of confidentiality:** As in any study, there is the potential for loss of confidentiality. Female subjects of childbearing potential will require urine pregnancy testing prior to enrollment in the protocol, and results of this testing have potential to result in loss of confidentiality.
- **Risk of glycemic deterioration:** It is possible that changing dietary requirements or insulin doses may result in glycemic deterioration. Likewise, adolescents may have glycemic deterioration due to non-compliance. During the study, dose adjustments will be made according to standard of care to prevent hypoglycemia and hyperglycemia.
- **Risk of CGM:** Participants have the option of using FDA approved CGM as part of clinical care in our clinics. There is a low risk of developing a local skin infection at the site of the sensor needle placement. Itchiness, redness, mild bleeding, and or bruising may occur at the insertion site. Subjects may develop localized reactions to adhesive used to secure the sensor.
- **Risk of study questionnaires**: Participants may experience some distress when discussing factors important to diabetes, diabetes management, and psychosocial stressors

Minimizing Risks:

- Risks of Very Low Carbohydrate Diet: In order to minimize the symptoms associated with transitioning to a low carbohydrate diet abruptly, participants will be encouraged to stay hydrated to help them acclimate and avoid unwanted symptoms.
- Hypoglycemia/Hyperglycemia: Participants will be contacted frequently during the course of the study. Blood draws will be done frequently during the HRU/CSRU/Masonicare admission to minimize hypoglycemia and hyperglycemia. Intravenous glucose will be available in case hypoglycemia occurs. All infusates containing insulin are prepared under a filtration cabinet (Laminated HLDA filter) by qualified personnel using sterile disposable materials.
- Participants will be encouraged to monitor closely for signs of glycemic deterioration and will be able to contact our diabetes staff at any time if concerns arise.
- At the end of each visit, the participant will receive guidance for glucose monitoring, food intake, and insulin dosing as needed.
- During the study, insulin doses will be adjusted regularly (2-3 times per week as needed) to prevent hypoglycemia. Participants will use a real-time CGM that will provide alerts at the time of hypoglycemia and allow for early detection of hypoglycemia as well. Participants will be trained on treatment of hypoglycemia and to contact the study team immediately in any case of severe hypoglycemia.
- If any participant has 2 episodes of severe hypoglycemia, they will be transitioned to a regular diet after the 2nd episode of severe hypoglycemia.
- Stable isotopes: Isotopes will be carefully monitored, administered and will be prepared in the investigational pharmacy to ensure proper technique. As noted in

previous protocols using this stable isotope, the pyrogenicity and sterility of the isotopes are documented by Cambridge Isotopes before they are shipped to YNHH IDS. Methods to Ensure Compound Sterility and Pyrogenicity: All stable isotopes are purchased from Cambridge Isotopes (Boston, MA) and are sent to us sterile and pyrogen-free as specified in the certification form of analysis that is attached to each order. In addition, all infusates containing the isotope that will be used in the study are prepared by the Yale Investigational Drug Service (IDS) by using specific filters (0.22μ). Furthermore, we also use microfilters at the end of each syringe as another safety precaution. All stable isotopes are stored by the Yale IDS and are sent to the YCCI the morning of the study.

- Risk of Indirect Calorimetry: Potential feelings related to wearing the "hood" will be discussed with participants ahead of time, if participants become uncomfortable while wearing the hood it will be removed.
- Risk of indwelling catheter: IV insertion will be performed by trained research nurses on the HRU/CSRU/Masonicare. Trained nurses will use aseptic technique to insert the IV catheters. We will minimize the risk of pain by offering an option of topical anesthetic to the patient prior to IV insertion. Participants will be advised to contact the study team immediately if they become aware of a late adverse effect.
- Anemia: Samples will be obtained with the minimum necessary blood volume. The point of care YSI glucose determinations require only 0.3ml of blood. We will ensure that blood volume obtained does not exceed a maximum of 5 mL/kg in pediatric participants and 250 mL in adult participants. We limited the lower limit of weight to 43 kg so as not to exceed blood draw volumes.
- Risk of loss of confidentiality: All study staff are HIPPA certified. Participant names and study records will be kept confidential. The IRB may inspect study records at any given time. All efforts will be made to maintain the participants' privacy. Each participant will be assigned a study ID code and data will be linked by this study ID rather than by names, initials, social security numbers, or other personal identifiers. Paper documents will be stored in locked areas accessible only to authorized staff. All electronic data files will be protected by passwords limiting access to the files only to those with a legitimate responsibility for data entry or management. This ensures that, in the unlikely event that any primary data sources from this study are lost or misplaced, it will not be possible to identify the study participant. Copies of informed consent/assent forms and families' contact information will be stored separately from the other study data, which will not contain names or other identifying information. Identifiable study information will be maintained for 10 years after the research is complete. After that time, it will be destroyed or de-identified. The principal investigator will keep a link that identifies subjects to coded information, but this link will be kept secure and available only to the PI or selected members of the research team.
- Risk of CGM: Risks of glucose sensor insertion will be minimized because participants will be instructed to cleanse skin aseptically prior to insertion. Participants will receive training on sensor use if they have not used the sensor previously.
- Risk of study questionnaires: Study staff and social work will be contacted to support families as needed.

3.2.2 Potential Benefits:

- Participants may experience improved glycemic control by participating in the study.
- Participants may learn more about how the macronutrient content of food can affect blood sugar levels.
- Long-term benefits of this research are a generalizable understanding of the changes of insulin resistance and counterregulatory hormone responses to

hypoglycemia relate to dietary changes for youth with T1D, which will help to inform future treatment strategies.

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4 Study Objectives

4.1 Hypotheses

Aim 1: We hypothesize that, compared to a standard carbohydrate diet, adolescents with T1D on a very low carbohydrate diet will spend more time in the target glucose range, will have a more robust response to hypoglycemia, and will lessen hypoglycemia awareness.
Aim 2: We hypothesize that counterregulatory hormone response to hypoglycemia (glucagon production) will be amplified in a very low carbohydrate diet when compared to standard carbohydrate diet. This will occur because of lower insulin doses on the very low carbohydrate diet that will translate to lower glycogen stores, and amplify the counterregulatory hormone response to hypoglycemia.
Aim 3: We hypothesize that hypoglycemia symptoms will be blunted on a very low carbohydrate when compared to a standard carbohydrate diet.

4.2 Primary Objective

The primary objective of this study is to determine whether youth with type 1 diabetes will improve glycemic control (increase time in the target glucose range of 70-180 mg/dL) following a very low carbohydrate diet when compared to a standard carbohydrate diet.

4.3 Secondary Objectives (if applicable)

The secondary objectives of this study are to compare the metabolic responses to hypoglycemia when adolescents are on a very low carbohydrate diet to a standard carbohydrate diet. We will also compare glucose and glycerol metabolism using stable isotope infusions between the two diets. We
will use a 2-step hyperinsulinemic euglycemic-hypoglycemic clamp. Nonradioactive stable isotope infusions of glucose and glycerol will be given during the clamp to measure changes in glucose and glycerol metabolism (hepatic glucose production and adipose tissue lipolysis).

4.4 Exploratory Objectives (if applicable)

We will compare symptoms of hypoglycemia during hyperinsulinemic hypoglycemic clamp in adolescents with T1D following a very low carbohydrate versus standard carbohydrate diet. Participants will report symptoms of hypoglycemia during euglycemia and hypoglycemia portions of each clamp to determine if hypoglycemia awareness is affected by each type of diet. Further, interviews will be conducted to collect descriptive qualitative data about participants' and parents' experiences following a very low carbohydrate diet.

5 Study Design

5.1 General Design Description: Currently, there are no specific dietary recommendations for adolescents with T1D. To fill this gap in knowledge, we will study a very low carbohydrate diet that limits carbohydrate intake to 15% of total daily calories. We hypothesize that, compared to a standard carbohydrate diet, adolescents with T1D on a very low carbohydrate diet will spend more time in the target glucose range, will have a more robust response to hypoglycemia, and will lessen hypoglycemia awareness. To test these, we will conduct a randomized crossover study to compare the metabolic and neurological responses to hypoglycemia in adolescents after following each isocaloric diet.

5.1.1 Study Date Range and Duration: 4 years

5.1.2 **Number of Study Sites**: The enrollment visit will take place at the Pediatric Diabetes Research Center at 1 Long Wharf Drive, Suite 503. The clamp procedures will take place at the CSRU, HRU, and Masonicare.

5.2 Outcome Variables

5.2.1 Primary Outcome Variables

Aim 1: The primary efficacy outcome will be the time spent in the target range of 70-180 mg/dL during the second week on each diet. Secondary safety outcomes will include the time spent in the hypoglycemic (less than 54 mg/dL, less than 70 mg/dL) and hyperglycemic (greater than 180 mg/dL, greater than 250 mg/dL) ranges. We will also measure the average sensor glucose values, standard deviation, and coefficient of variation during the second week on each diet period. We will compare average and range of fasting beta-hydroxybutyrate levels during each diet period. Number of episodes of diabetic ketoacidosis and severe hypoglycemic events during each period (baseline, diet periods, and washout period) will also be reported.

Aim 2: The primary safety outcome will be the mean levels of glucagon produced in response to hypoglycemia after each diet will be measured using a 2-step hyperinsulinemic euglycemic-hypoglycemic clamp. These will be measured after 15, 30, 45, and 60 minutes of euglycemia and hypoglycemia. Secondary safety outcomes will include mean levels of other counterregulatory hormones (cortisol, epinephrine, norepinephrine), and beta-hydroxybutyrate that are produced in response to hypoglycemia during the clamp will be compared after each 2-week diet period. Fasting levels of glucagon, free fatty acids, beta-hydroxybutyrate and IGF-1 (measure of growth hormone secretion), prior to the clamp will be compared after each diet period. Secondary outcome measures will include hepatic glucose production and adipose tissue lipolysis, which will be measured using stable isotope infusions during the euglycemic and hypoglycemic portions of the clamp. We will compare differences in hepatic glucose production during each clamp between a standard and very low carbohydrate diet. Additionally, deuterated water will be utilized to differentially evaluate the contribution of gluconeogenesis and glycogenolysis to endogenous glucose production.

Aim 3: The primary safety outcome will be hypoglycemia awareness symptoms that occur after 20-30 minutes of euglycemia and hypoglycemia during each clamp using a validated Edinburgh Hypoglycemia Symptom questionnaire. Scores during euglycemia will be compared to scores during hypoglycemia for each clamp. Scores during hypoglycemia after each diet period will also be compared. To describe how participants feel about and react to following a very low carbohydrate diet, interviews will be conducted over

zoom (HIPAA compliant). Data will be coded and emerging patterns will be combined into descriptive themes.

5.3 Study Population

Participants will be youth ages 12 and <25 with a diagnosis of type 1 diabetes for at least 1 year and have an A1c level < 10%. Participants will be recruited from the Yale Pediatric Diabetes Center and Yale Diabetes Center. Participants will also be recruited from ClinicalTrials.gov and social media (Facebook, Twitter, Instagram). The Yale Pediatric Diabetes Center Clinics, which sees over 1000 pediatric patients each year. Of those, 425 patients are between the ages of 12 and 18 with type 1 diabetes each year. It is estimated that 255 (60%) in this age group have an A1c level of < 10% and will be eligible for the study. Based on this information, 85 potential participants are seen each month in our clinics. We anticipate being able to recruit at 10% (8-9 participants) of these patients each month, which is more than enough to complete the study. All participants will undergo pre-screening of their electronic medical record to evaluate study eligibility by review of age and previous laboratory tests. Flyers will also be distributed in the clinics. Participants who meet eligibility criteria may also be contacted by MyChart. Patients who have already completed the study will be contacted about follow up interviews that will be voice-recorded over zoom.

5.4 Number of Participants: 20 total participants will be needed to complete the study.

5.4.1 Eligibility Criteria/Vulnerable Populations: The principal investigator (Laura Nally) will determine eligibility and inclusion/exclusion criteria.

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Provision of signed and dated informed consent form by the adult (if person is at least 18 years old) or parent and assent form if the participant is <18 years old.
2. Stated willingness to comply with all study procedures and availability for the duration of the study
3. Male, female, transgender, nonbinary are all eligible
4. Ages 12 to <25 years, inclusive, of all races/ethnicities
5. Diagnosis of type 1 diabetes for at least 1 year
6. Hemoglobin A1c level < 10% within the past 2 months
7. Insulin pump use for at least 2 months.
8. Ability to perform daily blood ketone checks, wear a continuous glucose monitor, willing to wear an activity monitor, willing to adhere to the study diets and keep a diet journal.
9. For females of reproductive potential: use of highly effective contraception for at least 1 month prior to screening and agreement to use such a method during study participation and for an additional 4 weeks after the end of the dietary intervention.
10. For males of reproductive potential: use of condoms or other methods to ensure effective contraception with partner

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current use of glucose-lowering agents other than insulin
2. Unstable psychiatric disorders, including eating disorders (DSM-V criteria).
3. Weight loss medications within the last 6 months
4. Pregnancy or lactation, or planning pregnancy within the next 6 months
5. BMI < 19 kg/m2 for individuals 18 years of age or older

6. BMI < 5%ile for individuals under 18 years
7. Weight less than 49 kg or 108 pounds (due to blood draw)
8. Other medical conditions that may interfere with glucose metabolism and insulin sensitivity (as determined by the investigator).

6 Methods

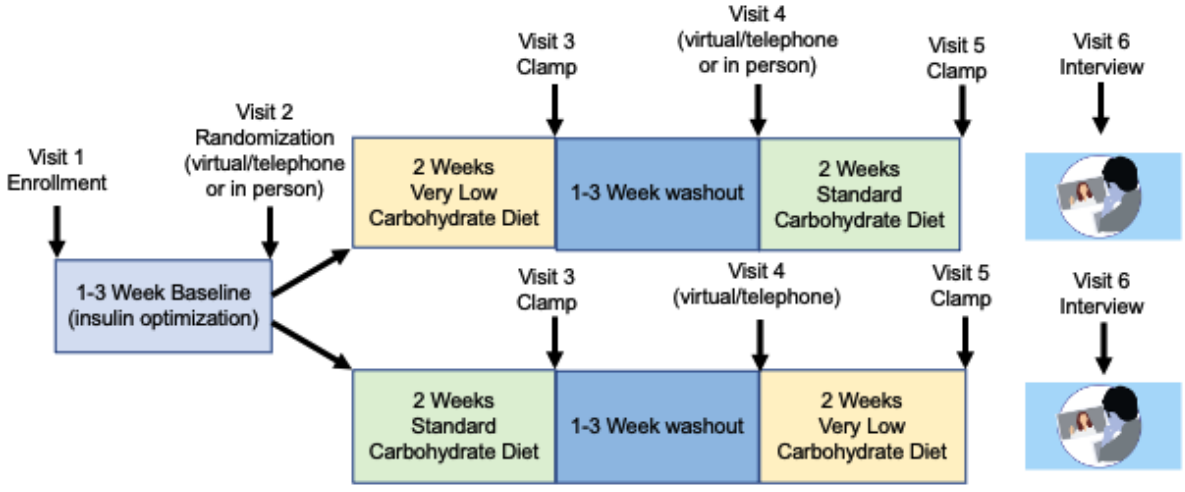
6.1 Treatment

6.1.1 Identity of Investigational Product – N/A

6.1.2 Dosage, Administration, Schedule

Visit 1 will consist of informed consent and enrollment. The study will begin with a 1 to 3 -week baseline period for insulin optimization. At visit 2, participants will be randomized for either the very low carbohydrate diet or standard carbohydrate diet that they will follow for 2 weeks. At visit 2 (virtual/telephone/in person), participants will be instructed on how to keep diet journal and report relative amounts of food during the 24-hour recall phone calls. After 2 weeks on the first diet, participants will come to the HRU/CSRU/Masonicare for Visit 3, the hyperinsulinemic euglycemic-hypoglycemic clamp. Following this visit, the participants will have a 1-3 week washout period. At Visit 4 (virtual/telephone/in person), the next diet will be reviewed. They will follow that diet for 2 weeks and then return to the HRU/CSRU/Masonicare for Visit 5 and the second hyperinsulinemic euglycemic-hypoglycemic clamp. After this visit, the study will be complete.

Figure 1. Schedule of visits during the study.



6.1.3 Method of Assignment/Randomization

Block randomization will be used to ensure an equal number of participants complete diets in each of the specified sequences.

6.1.4 Blinding and Procedures for Unblinding – N/A

6.1.5 Packaging/Labelling

There is no study drug for this protocol. However, stable isotopes will be obtained from Cambridge Isotopes, Cambridge, MA), which is routinely audited internally by its QA department and externally by customers, notified bodies, and regulatory

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agencies (e.g. FDA). Isotopes will be stored in the Investigational Pharmacy and will be prepared before the subjects undergo tests. Deuterated water will also be obtained from Cambridge Isotope Labs.

6.1.6 Storage Conditions

The glycerol and glucose isotopes, dextrose, and insulin infusions will be kept with Yale-New Haven Hospital Investigational Drug Service (IDS), which will keep all documentation. The IDS will prepare preparations of isotopes before each study and store it in the med room in the YCCI. Pyrogen and sterility are tested and documented by Cambridge Isotopes before shipping to us.

6.1.7 Concomitant therapy

As is stated in the exclusion criteria, no medications (other than insulin) that cause glucose lowering will be permitted during the study.

6.1.8 Restrictions- N/A

6.2 Assessments

6.2.1 Efficacy

Aim 1	
Primary Efficacy Outcome	Percent time spent in the target range of 70-180 mg/dL during 2 weeks on each diet, as measured by CGM.
Secondary Safety Outcomes	We will measure the percent time spent in the hypoglycemic (<54 mg/dL, <70 mg/dL) and hyperglycemic (>180 mg/dL, >250 mg/dL) ranges. We will also measure the average sensor glucose values, standard deviation, and coefficient of variation during each 2-week diet period. We will compare the average fasting β-hydroxybutyrate levels during each diet period. Number of episodes of diabetic ketoacidosis and severe hypoglycemic events during each period (baseline, diet periods, and washout period) will also be reported.
Aim 2	
Primary Safety Outcomes	Mean levels of glucagon produced in response to hypoglycemia after each diet will be measured using a 2-step hyperinsulinemic euglycemic-hypoglycemic clamp. These will be measured every 15-30 minutes of during the first step of the euglycemic clamp and every 15 minutes during the hypoglycemic clamp.
Secondary Safety Outcomes	Measure mean levels of other counterregulatory hormones (cortisol, epinephrine, norepinephrine) and β-hydroxybutyrate that are produced in response to hypoglycemia during the clamp will be compared after each diet period. Fasting levels of glucagon, free fatty acids, β-hydroxybutyrate and IGF-1 (measure of growth hormone secretion) prior to the clamp will be compared after each diet period.
Secondary Efficacy Outcomes	Hepatic glucose production and adipose tissue lipolysis will be measured using stable isotope infusions during the euglycemic and hypoglycemic portions of the clamp. We will compare differences in hepatic glucose production during each clamp between a standard and very low carbohydrate diet.

Aim 3	
Primary Safety Outcome	We will measure hypoglycemia awareness symptoms after 20-30 minutes of euglycemia and hypoglycemia during each clamp using a validated Edinburgh Hypoglycemia Symptom questionnaire. Scores during euglycemia will be compared to scores during hypoglycemia for each clamp. Scores during hypoglycemia after each diet period will also be compared.
Primary Eficacy Outcome	To describe how participants feel about and react to following a very low carbohydrate diet, interviews will be conducted over zoom (HIPAA compliant). Data will be coded and emerging patterns will be combined into descriptive themes.

6.2.2 Safety and Pregnancy-related policy

- Women of childbearing age will have a urine pregnancy test performed at the time of the screening/enrollment visit and the need for contraception for the entire duration of the study will be discussed. Additional urine pregnancy tests will take place the morning prior to each clamp visit (visit 3 and visit 5).
- Hematocrit levels will be tested prior to the clamp procedures (visit 3 and visit 5). Samples will be obtained with the minimum necessary blood volume. The point of care YSI glucose determinations require only 0.3 ml of blood per measurement. We will ensure that blood volume obtained does not exceed a maximum of 5cc/kg in pediatric participants and 550 ml in adult participants
- Continuous glucose monitoring data will be reviewed twice per week (or more frequently as needed) to ensure participants are wearing the devices and to monitor for hyperglycemia and hypoglycemia. Insulin doses will be titrated by trained research staff based on clinical care standards to ensure safety by minimizing hypo- and hyperglycemia.
- Daily fasting blood ketone checks will be performed during the study intervention and as needed for symptoms of ketosis. If ketones are elevated, participants will be instructed to call the diabetes emergency line 24/7 for further instruction. All patients in our clinics are instructed to call this number if they develop elevated ketones at home. These values will also be reviewed 3 times per week when 24-hour dietary recall is performed.

6.2.3 Adverse Events Definition and Reporting

Definitions
Adverse event (AE) means any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)).
An AE or suspected adverse reaction is considered "serious" (SAE) if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- a congenital anomaly/birth defect, or

- An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Severity
Adverse events will be graded according to CTCAE v5.0.. For AEs not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- Mild – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- Moderate – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating.

Relationship to Investigational Product
All AEs must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

- Definitely Related – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- Probably Related – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- Potentially Related – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.

- Unlikely to be related – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- Not Related – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

Expectedness
The Principal Investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

6.2.4 Pharmacokinetics (if applicable) – N/A

6.2.5 Biomarkers (if applicable) – N/A

6.3 Study Procedures

6.3.1 **Study Schedule:** See figure 1. The study will consist of 5 visits: The enrollment visit, randomization, and 2 visits for clamp procedures following each diet and a virtual/telephone follow up visit.

Enrollment Visit 1	Screening and Consent. Discuss research procedures. Meet with a registered dietician or trained research staff to discuss diet and education on keeping a diet record. Review of insulin pump and CGM data.
Randomization Visit 2 (Virtual/Telephone or in person)	Randomization will occur, as will discussion of subsequent diet (and how to obtain meals), study requirements, and next steps. Review of insulin pump and CGM data.
Clamp Visit 3	Hyperinsulinemic-euglycemic 2-step clamp followed by hypoglycemic clamp. Study questionnaires. Review insulin pump and CGM data. Discussion of washout period and next steps. Review of insulin pump and CGM data
Visit 4 (Virtual/Telephone or in person)	Discussion of subsequent diet (and how to obtain meals), study requirements, and next steps. Review of insulin pump and CGM data.
Clamp Visit 5	Hyperinsulinemic-euglycemic 2 step clamp followed by hypoglycemic clamp. Study questionnaires. Review of insulin pump and CGM data. Discussion of potential need for insulin dose changes and follow up.
Visit 6	Interviews will be conducted over the telephone or over zoom.

6.3.2 Informed Consent:

- Written informed consent will be obtained from legally authorized representatives for those under age 18 prior to conducting any study-related procedures.
- Written informed consent will be obtained from individuals at least 18 years of age.
- For those under age 18, written informed adolescent assent will be obtained from adolescents.
- Participants and legally authorized representatives will be given time to think about the study and ask questions as needed. We will discuss that non-participation in the study will not affect clinical care to minimize undue influence or coercion.

6.3.3 Screening

- Participants will be recruited from the Yale Pediatric Diabetes Center and Yale Diabetes Center clinics using flyers or by recommendation of their primary clinician. Participants will undergo pre-screening of their electronic medical record to evaluate study eligibility by review of medical history, as well as prior laboratory tests
- Participants will be recruited using social media (Facebook, Instagram, Twitter).
- Participants may reach out to the investigators after reading about the study on ClinicalTrials.gov.
- Participants who are not in the Yale Health System will be asked screening questions over the phone prior to the enrollment visit.
- If it has been longer than 2 months since the most recent HbA1c level, participants will be re-screened by obtaining a HbA1c at the pediatric diabetes research center.
    - **Request for waiver of HIPAA authorization for recruitment/screening purposes only**
    - We will need to review the potential subject's medical record prior to approaching them about the study in order to confirm that they are medically eligible. It is impractical to approach patients about the study if they are not medically eligible, given the time involved in approaching patients and the volume of patients seen at the various clinics. Once we have determined initial medical eligibility, we will then screen those patients in-person, or over the phone if patients have agreed to be contacted in this manner, to confirm all eligibility criteria has been met.
    - **Recruitment Methods.** Potential subjects will be identified in several ways:
      1. At the time of routine, clinical visits with study investigators and their colleagues. They may be approached in-person about the study by clinical staff and/or research staff. This initial contact may be followed up with a phone call, email, letter or MyChart message if the patient is willing to speak to a research team member.
      2. Recruitment materials posted in the community and on social media (Twitter, Facebook, Instagram) via YCCI and/or study personnel accounts. The flyer may also be distributed to the YCCI "Help Us Discover" database of more than 12,000 individuals who have expressed interest in clinical research at Yale. Emails will be sent to this database periodically about the study. Information about the study will also be posted on the YCCI's website. In addition, the research team will enlist the help of the YCCI Cultural Ambassadors to advertise for the study at community organizations and events utilizing the recruitment flyer.
      3. Direct-to-patient recruitment via the Joint Data Analytics Team (JDAT). This is a behind-the-scenes electronic medical record search run by JDAT for the Yale New Haven Health System (YNHHS). This search will not identify potential participants to the researchers. Once JDAT identifies eligible patients, these

potential participants will be sent a message via MyChart. Potential subjects who do not use MyChart will receive a paper mailing. If no response is received, the YCCI Recruitment Center may follow-up with a phone call to determine interest. We will also utilize JDAT to generate weekly schedule reports of eligible patients being seen at Pedi and Adult Endo clinics.

- The following wording will be used in the email, letter or MyChart message patients receive via JDAT and otherwise describing the study and inviting them to participate:

"You are receiving this [email or message or letter] because you have type 1 diabetes and are at least 12 years old. You may be eligible to participate in a free and confidential research study investigating metabolic changes that occur on a high protein/low carbohydrate diet. This is an 8 week study with 6 study visits. In order to participate, you must:

- Be between the ages of 12 to <25 years old
- Have type 1 diabetes for at least 1 year
- Use an insulin pump for at least 2 months
- Have a HbA1c level < 10%
- Weigh at least 108 pounds
- Be willing to wear a continuous glucose monitor for the duration of the study
- Be willing to keep a daily diet record and follow dietary guidelines

During the study you will be provided with:

- 3 meals per day during the very low carbohydrate diet (2 weeks)
- Continuous glucose monitoring supplies for the duration of the study (if you do not already have one)
- Blood ketone meter and strips (you can keep these)
- Activity monitor and scale (you can keep these)
- Compensation is up to $420

To learn more or to see if you are eligible to participate, click on "I am interested" or call the 'Help us Discover' recruitment call center at 1-877-978-8343. This message is automated and is sent in an electronic manner based on your health record; no one has been inside or viewed your medical chart. No action by you is required. You may ignore this message or click "not interested." Thank you very much for considering being a part of research at Yale. To learn about future research opportunities, you may also create a volunteer profile through the Research Tab in MyChart. To opt-out of all future research communications, please call the 'Help us Discover' recruitment call center at 1-877-978-8343 and select #3."

6.3.4 Enrollment

- Participants will be enrolled by research study staff identified in this protocol.
- After confirming that screening criteria have been met and informed consent has been obtained and documented, subjects will undergo a history and directed physical exam, anthropometric measures, and hemoglobin A1c. A HbA1c done in the past 2 months may be used.

6.3.5 On Study Visits

Visit 1 (Screening/Enrollment):

- Screening for inclusion/exclusion criteria
- Written informed Consent/Assent completion prior to any study activities
- Participants will be asked permission to allow the serum and information collected during this research study to be used for future research purposes. The serum samples may be used by our research group to run additional assays related to the pathophysiology of diabetes.
- Demographic characteristics including education level, family income, number of individuals in the home
- Current diabetes care practices (frequency of self-monitored blood glucose (SMBG), insulin dosing, review of blood glucose values)
- Anthropomorphic measures (height, weight, BMI, vital signs)
- Physical exam, tanner staging
- Urine pregnancy test (if participant is post-menarche)
- Point of care HbA1c, obtained via fingerstick and measured by a Seimens DCA Vantage device, will be determined unless an A1c was measured in clinic within the past 30 days.
- Medical History: duration of diabetes, comorbidities, past medical history, social history, family history, medications, and allergies.
- Additional diabetes history including current/past use of CGM, insulin pump, and other modes of diabetes technology.
- A handout with 2 examples of the type of diet required will be provided to each participant at the time of or prior to consent.
- All participants will receive dietary instruction (by a registered dietician or trained research staff) to review the dietary requirements. Each participant will be instructed on how to keep a food diary, estimate food portions and receive instructions on information required during dietary recall.
- Participants will meet with a diabetes care provider (i.e., a physician, nurse practitioner, or physician assistant) who will carry out a comprehensive review of diabetes management and review blood glucose and insulin dosing records. Insulin pumps and CGMs will be downloaded to assess glycemic control. The diabetes treatment regimen will be adjusted as clinically indicated.
- A CGM will be provided if the individual does not have one that they use as a part of routine care. Participants will be instructed on how to use these devices, as they would typically be instructed at clinic visits.
- CGM low alerts will be set at 55 mg/dL and 70 mg/dL. CGM high alerts will be individualized to each patient, but be kept consistent during both study dietary periods.
- Participants will receive an activity monitor (Fitbit or Garmin) if they do not already have one and instructed on its use. We will ask participants to maintain a consistent activity level throughout both dietary periods. You may keep the activity monitor (Fitbit or Garmin) that we provide for you.
- Participants will also receive a bioimpedance scale to use at home for anthropomorphic measurements.
- Participants will receive a blood ketone meter and ketone strips to use for the duration of the study.
- Questionnaires will be administered using Qualtrics or paper/pen, including:
    - Diabetes Treatment Satisfaction Questionnaire (DTSQ), which includes eight items, six of which form a scale (scored 0-36) in which higher scores indicate greater treatment satisfaction. (11)
    - Nutrition Knowledge Survey (NKS) which collects information on healthful eating, carbohydrate counting, blood glucose response to foods, and nutrition label reading. Higher NKS scores reflect greater nutrition knowledge (score range is 0–100%). (12)

- Clarke Hypoglycemia awareness questionnaire.
- Open-ended questionnaire regarding the diet (see Additional Documents)

Follow Up Calls:

- Participants will be followed up weekly to make insulin dose adjustments during the insulin optimization and washout periods. Insulin pump and CGM information will be reviewed during these phone calls.
- Participants will also be screened for any symptoms that may be related to very low carbohydrate diets on a weekly basis or more often as needed (symptoms including nausea, vomiting, abdominal pain, constipation, fatigue, and lightheadedness). If these occur, participants will be instructed to ensure adequate caloric intake is achieved and hydration is maintained.

Visit 2 Randomization (virtual/telephone or in person):

- Anthropomorphic measures (weight, % body fat, % muscle mass, % water, % bone) – home or on scale in office.
- Insulin pumps and CGMs will be downloaded to assess glycemic control. The diabetes treatment regimen will be adjusted as clinically indicated.
  - If participants are starting the very low carbohydrate diet, insulin doses will be reduced by 10-20%, as clinically indicated, to prevent hypoglycemia.
- First diet will be assigned.
  - If participants are starting the very low carbohydrate diet, meals can be picked up 2-3 times per week at the metabolic kitchen located at Yale New Haven Hospital by study participants or by research staff. Research staff will work with participants to deliver the meals if they are unable to pick them up.
  - If we are unable to acquire meals from the metabolic kitchen, we will use a mail order meal service that provides information about macronutrient content of all meals provided that meet the standards of the dietary measures.
- Participants will be instructed to check daily fasting blood ketone levels.
- Participants will receive dietary instruction and review the dietary requirements of the study. Participants will be instructed to keep a dietary record (MyFitnessPal application, written, and/or with photos) for the duration of the dietary period.
- Participants will be instructed to call the pediatric diabetes emergency line (available 24/7) in any case of severe low blood sugar, elevated ketone levels, or symptoms of ketosis. Participants will also receive education on the treatment of hypoglycemia, we will ensure that all participants have glucagon at home.
- Participants will be contacted 3 times per week (2 scheduled, 1 unscheduled) for a 24-hour dietary recall. During these telephone follow up calls, food diary will be reviewed, ketone levels reviewed, and CGM data will be uploaded and reviewed.
- Prior to the HRU/CSRU/Masonicare admission, we will recommend a standard diet be given and no food be eaten after 9pm (except for in cases of hypoglycemia). Participants will be advised to limit exercise in the 3 days prior to study to limit acute effects of recent exercise on insulin sensitivity.

Visit 4 (virtual/telephone or in person):

- Anthropomorphic measures (weight, % body fat, % muscle mass, % water, % bone) – at home or on scale in office
- Insulin pumps and CGMs will be downloaded to assess glycemic control. The diabetes treatment regimen will be adjusted as clinically indicated.
  - If participants are starting the very low carbohydrate diet, insulin doses will be reduced by 10-20%, as clinically indicated, to prevent hypoglycemia.
- Second diet will be assigned.

  - If participants are starting the very low carbohydrate diet, meals can be picked up 2-3 times per week at the metabolic kitchen located at Yale New Haven Hospital by study participants or by research staff. Research staff will work with participants to deliver the meals if they are unable to pick them up.
  - If we are unable to acquire meals from the metabolic kitchen, we will use a mail order meal service that provides information about macronutrient content of all meals provided that meet the standards of the dietary measures.
- Participants will be instructed to check daily fasting blood ketone levels.
- Participants will receive dietary instruction and review the dietary requirements of the study. Participants will be instructed to keep a dietary record (MyFitnessPal application, written, and/or with photos) for the duration of the dietary period.
- Participants will be instructed to call the pediatric diabetes emergency line (available 24/7) in any case of severe low blood sugar, elevated ketone levels, or symptoms of ketosis. Participants will also receive education on the treatment of hypoglycemia, we will ensure that all participants have glucagon at home.
- Participants will be contacted 3 times per week (2 scheduled, 1 unscheduled) for a 24-hour dietary recall. During these telephone follow up calls, food diary will be reviewed, ketone levels reviewed, and CGM data will be uploaded and reviewed.
- Prior to the HRU/CSRU/Masonicare admission, we will recommend a standard diet be given and no food be eaten after 12am (except for in cases of hypoglycemia). A snack will be recommended at 9pm the evening prior to the clamp. Participants will be advised to limit exercise in the 3 days prior to study to limit acute effects of recent exercise on insulin sensitivity.

HRU/CSRU/Masonicare (Visits 3 & 5):

- Participants will arrive the evening prior to the study or the morning of the study at 7am.
- Anthropomorphic measures (height, weight, BMI, vital signs)
- Urine pregnancy test (if participant is post-menarche)
- Insulin pumps and CGMs will be downloaded to assess glycemic control. The diabetes treatment regimen will be adjusted as clinically indicated.
- Questionnaires will be administered using Qualtrics, including:
  - Diabetes Treatment Satisfaction Questionnaire (DTSQ), which includes eight items, six of which form a scale (scored 0-36) in which higher scores indicate greater treatment satisfaction (Bradley, 1994).
  - Open-ended questions about the diet.
- Two indwelling catheters will be placed, one into an antecubital vein for infusion of test substances and a second catheter into a contralateral vein for blood sampling. Blood draws for measurements of glucose and glycerol enrichment are listed in tables 3, 4, 5, and 6.
- Baseline laboratory assessments will include:
  - Glucose (YSI)
  - Free fatty acid levels
  - Glucagon
  - Beta-hydroxybutyrate
  - IGF-1 (measure of growth hormone secretion)
  - Hemoglobin A1c (if none obtained in the past 30 days)
- Visit 6:
  - Participants will meet with study staff over the telephone or over HIPAA-compliant Zoom. Interviews will be conducted (see interview script) in a private, confidential place and recorded using Otter software (https://otter.ai) that involves live transcription. Participants and their parents will be asked open-ended questions about their experiences following a very low

carbohydrate diet. Participant and parent interviews will be conducted together, however participants will also be given the opportunity to share their thoughts without parents present at the end of the interview. Interviews will last about 20-30 minutes.

Figure 2. Schedule of HRU/CSRU/Masonicare measurements.

Baseline glucose and glycerol turnover	Hepatic sensitivity to insulin (low dose insulin)	Peripheral sensitivity to insulin (high dose insulin)	Hypoglycemia
150-minute equilibration	Euglycemic Low Dose (90 minutes)	Euglycemic High Dose (120 minutes)	Hyperinsulinemic Hypoglycemic Clamp (60 minutes)

- Equilibration Period*:* The study will begin with a ~150 minute equilibration period with a stable isotope infusion during which we will aim to keep glucose levels stable. Non-radioactive, Stable Isotope Tracer Infusions will be employed to assess rates of glucose and glycerol metabolism at the end of the ~150-minute baseline equilibration period. From -150 to 0 min, 4.5 mg/kg of 6,6-$^{2}H_2$-glucose (99% enriched; Cambridge Isotope Laboratories, Andover MA) will be followed by a continuous infusion at 0.06 mg/kg/min. $^{2}H_5$- glycerol will be infused at 1.6 µmol/kg followed by a constant infusion of 0.11 µmol/kg/min to assess glycerol turnover. During the basal turnover period, a variable rate insulin infusion may be utilized to maintain plasma glucose between ~90-110 mg/dL. As results from prior participants become available, infusions of the isotopes may be adjusted in subsequent participants to ensure enrichment is not too low for measurement.
- Oral Non-radioactive, Stable Isotope Tracer*:* 3 ml/kg body water of deuterated water will be given orally in 3 divided doses, (1 ml/kg body water in the evening, 1 ml/kg body water overnight, and 1 ml/kg body water early morning). The total D2O given (3 ml per kg body water) is designed to raise body deuterium levels to 0.3% and is administered as described above to maintain steady state until the end of the study. An additional 4.5ml mixed in 1000 ml tap water will be available to participants to drink for thirst, however this is not required.
- Low Dose Insulin Infusion Period to Assess Hepatic Sensitivity to Insulin: After obtaining baseline samples, the low dose insulin (8 mU/M2 surface area/min) infusion will be added to the basal insulin requirement for 90 minutes along with a variable 20% dextrose infusion with a goal of maintaining plasma glucose at 90-95 mg/dL. Plasma glucose levels will be measured every 5 minutes at the bedside throughout the clamp procedure.
- High Dose Insulin Infusion Period to Assess Peripheral Sensitivity to Insulin*:* The high dose insulin infusion of 80 mU/M2 surface area/min will also continue for 120 minutes accompanied by a variable 20% dextrose infusion.(14) Plasma glucose will be monitored every 5 minutes during both insulin infusion periods by the YSI 2300 glucose analyzer to adjust dextrose infusion rates to maintain plasma glucose 90-95 mg/dL.
- Measures of Oxidation*:* Indirect calorimetry will be utilized at the end of the baseline equilibration period, low dose, and high dose insulin euglycemic insulin infusions to evaluate rates of glucose and fat oxidation.
- Hyperinsulinemic hypoglycemia period to assess counterregulatory hormone responses*:* At the end of the hyperinsulinemic euglycemic portion of the clamp, the insulin infusion will be reduced to 40 mU/M2 surface area/min and exogenous glucose infusion will be reduced to allow the plasma glucose to fall to ~55 mg/dL where it will be clamped for an additional 60 minutes. After this time, the insulin

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infusion will be stopped and euglycemia restored by increasing the intravenous glucose infusion at the end of the study. Plasma glucose levels will be measured every 5 minutes at the bedside throughout the clamp procedure. Once euglycemia is restored, participants will be given a meal and given instructions for the next portion of the study prior to discharge home. The Edinburgh hypoglycemia questionnaire will be conducted by asking the participants questions after 20-25 minutes of hypoglycemia.

- Metabolite collection: Samples for measurements of glucose and glycerol enrichment as well as hormones and substrates will be obtained every 5-10 minutes during the baseline equilibration phase as well as during the final 30 minutes of low dose, high dose, and hypoglycemic phases. Blood samples collected as a part of this research will be stored by the YCCI core lab services.
- During the 2-step hyperinsulinemic euglycemic clamp, laboratory blood tests will include:
    - Glucose (YSI)
    - Insulin and free fatty acid levels
    - Glucagon levels
    - Stable isotope measurements
- During the euglycemia (after 0, 30, 45, 60 minutes of euglycemia) and hypoglycemia clamp (after 15, 30, 45, and 60 minutes of hypoglycemia), laboratory blood tests will include:
    - Glucose (YSI)
    - Epinephrine
    - Norepinephrine
    - Glucagon
    - Cortisol
    - Beta hydroxybutyrate
- During the hypoglycemia portion of the clamp, participants will be blinded to their glucose levels in order to collect information about hypoglycemia symptoms.

Dietary Records: Dietary records will be input into the ASA24 Dietary Assessment Tool (https://asa24.nci.nih.gov). The ASA24 application will calculate the daily macronutrient content ingested by each participant throughout the study period.

Insulin Pump/CGM records: Insulin pump and CGM data will be uploaded (Tidepool, Dexcom, Medtrnoic, Omnipod or Tandem site, depending on the insulin pump being used) and reviewed virtually, as is commonly done in clinical practice. Patients are trained on how to upload their pump and CGM data to the Tidepool (HIPAA compliant) website and videos are available online explaining how to upload these devices (https://medicine.yale.edu/pediatrics/endocrinology/cdp/media-player/5619/).

Interview Records: Interviews will be conducted by study staff over HIPAA compliant zoom or by telephone and recorded using Otter software. All interviews will be audio-recorded and transcribed verbatim and any identifying information will be removed from the transcript. Interview scripts will be used as a guide and evolve as the study progresses. Transcriptions will be reviewed for accuracy and coded using NVivo.

6.3.6 End of Study and Follow-up

- Participants will be instructed to contact study staff and make them aware of any adverse events that occur during the study (severe hypoglycemia, diabetic ketoacidosis, etc). Information about adverse events will also be asked at each study follow up visit.
- Upon completion of the clamp procedures, participants will be instructed to monitor their glucose levels carefully and contact clinic staff if they note that

insulin adjustments are needed. Follow up appointments with a diabetes care provider will be recommended within 2 weeks of the end of the study. Their primary diabetes provider will also be contacted and made aware that they have completed the study.

- If participants withdraw from the study early, participants will be instructed to monitor their glucose levels carefully and contact clinic staff if they note that insulin adjustments are needed. Follow up appointments with a diabetes care provider will be recommended within 2 weeks of ending the study. Their primary diabetes provider will also be contacted and made aware that they have withdrawn from the study.
- Diabetes providers will be made aware of any adverse events that take place during the study or information that is considered medically necessary for their overall health. Participants will be made aware of any information that may be shared with their diabetes provider. There is no plan to share results with the primary diabetes providers unless something pertinent to their diabetes management occurs during the study.

6.3.7 Removal of subjects: Participants may be withdrawn if they are unable to follow the dietary requirements and study procedures, including keeping a diet journal, testing ketone levels, and wearing a CGM at least 75% of the time.

6.4 Statistical Method

6.4.1 Statistical Design: see below.

6.4.2 Sample Size Considerations

For all aims, we base our sample size on the unadjusted comparison of paired means, which is a conservative approach. Therefore, given that our statistical analyses will be based on adjusted models and models that specifically take into account within-person correlation in outcomes of interest, we should be sufficiently powered to answer our research questions.

Aim 1: The goal time in range in adolescents with T1D 60% +/- 10% of their time in the target range of 70-180 mg/dL but based on our preliminary work in this population we expect that the mean percent of time in range is around 50-55%. With 16 eligible subjects, we will have 80% power at the two-sided alpha of 0.05 to detect a minimum increase in the expected mean by 7.5% (from 50-55% on standard carbohydrate diet to 57.5-62.5% on low carbohydrate diet - an increase of 1.5 hours per day - an effect size of 7.5%÷10%=0.75), using a paired-sample t-test. If we assume 20% attrition, we will need 20 individuals for the study.

Aim 2: Using data from a previously published study, where changes in glucagon levels during 2-step euglycemic-hypoglycemic clamp in youth with T1D were assessed over a period of 1 year(15), we anticipate that with 16 subjects (n=20 with 20% attrition, see aim 1) we will have 80% power at the two-sided alpha of 0.05 to detect a between-diet change in mean glucagon levels of at least 9.5 pg/mL (standard deviation=12.5 pg/mL, effect size of 9.5 pg/mL/12.5 pg/mL= 0.75), using a paired t-test. We will also report the incremental rise and area und the curve (AUC) as well as mean and peak glucagon levels.

Aim 3: Using estimates of variability (standard deviations [sd]=5 during euglycemia and sd=10 during hypoglycemia) from a previous study, with 16 subjects (20 subjects with 20% attrition) we will have 80% power at the two-sided alpha of 0.05 to detect an effect size in the range of 0.75-1.0, using a paired t-test, meaning that when on low carbohydrate diet, subject

will have on average higher awareness of hypoglycemia (e.g., at least 5-10 point difference in the mean total score) than when on standard diet. For interviews, following qualitative description methodology, data will be coded, and emerging patterns will be combined into descriptive themes. Thematic data analysis will proceed concurrently with data collection. Any differences in coding among researchers will be discussed until resolution is achieved. Emerging themes will be examined within and across interviews. Data coding and analysis will be conducted concurrently with ongoing data collection until emerging patterns and themes. Analyses will be conducted using a consensus model approach. All analytic decisions will be recorded in research memos or detailed notes of observations during data collection. The rationale for coding decisions and conceptual or theoretical issues that emerge during data analysis will be kept so as to maintain an audit trail during the data collection through the process of analysis. The research team has experience in qualitative methods and thematic analysis and those less experienced have received adequate training prior.

6.5 Planned Analyses

6.5.1 Primary Objective Analysis

- **Statistical Analyses Aim 1:** The unadjusted between-diet-group comparison of the primary endpoint, percent time in the target range of 70-180 mg/dL during the 2nd week of each diet, will be performed using a two-sided paired t-test. The adjusted analysis will be performed using linear mixed effects (LME) modeling, with a random intercept for each subject, diet effect, and adjusting for a participant's age, BMI and duration of diabetes. The mean adjusted between diet-group difference in percent time in the target range will be reported, along with the surrounding 95% confidence intervals based on 500 bootstrapped samples (2.5th percentile and 97.5th percentile of the distribution). Secondary outcomes, including time spent with hypoglycemia (<54 mg/dL, <70 mg/dL) and hyperglycemia (>180 mg/dL, >250 mg/dL), average sensor glucose level, will be analyzed similarly to the primary outcome. The proportion of days with elevated fasting β-hydroxybutyrate levels (≥0.6 mmol/L) during each diet period will be compared using paired-proportions z-test in the unadjusted analysis and using generalized linear mixed effects (GLMM) modeling (a random intercept, link=logit for the binary outcome yes/no elevated levels with Bernoulli distribution) with covariates of age, BMI, and duration of diabetes. GLMM will also be utilized in the comparison of the number of episodes of diabetic ketoacidosis and severe hypoglycemic events during each period (a random intercept, link=log for a count outcome with Poisson distribution). Statistical analyses for all aims will be implemented using R and SAS 9.4 (Cary, NC) statistical software. Statistical significance will be established at the two-sided alpha of 0.05.

6.5.2 Secondary Objectives Analyses

- **Statistical Analysis Aim 2:** Appropriate mathematical transformations (e.g., natural log or square root) will be applied to each outcome of interest, as necessary, to normalize the distribution. A paired t-test will be used in the unadjusted comparison of the primary outcome, mean levels of glucagon, during each diet period. LME will be used to model the trajectories of change in mean levels of glucagon over time (baseline, 15, 30, 45, 60 minutes) during each diet period and compared between diets. We will use the Likelihood Ratio Test (LRT, a mixture of two chi-square distributions with restricted maximum likelihood – RML - estimation) to select between an LME model with just a random intercept or an LME with a random intercept and slope. The mean response will be modeled using parametric (linear and polynomial effects of time) and semi-parametric (piece-wise linear or quadratic splines) effects of time, adjusting for participants' age, BMI, and duration of diabetes. The best model will be chosen based on its fit to the data (LRT for nested models and Akaike Information Criterion, AIC, for not-nested models).

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Effect sizes will be reported as the between-diet differences with bootstrapped 95% CIs. Secondary outcomes of interest will also be modeled similarly.

- **Statistical Analysis Aim 3:** Using the validated Edinburgh Hypoglycemia Symptom questionnaire, participants will be asked to verbally rate their hypoglycemia symptoms on a 7-point Likert scale (lower scores indicate fewer symptoms) during the euglycemia and hypoglycemia portions of the clamp. In the unadjusted analyses, differences between euglycemic and hypoglycemic total scores on each diet will be compared using a paired t-test. Adjusted analyses will be based on LME, similar to the analyses described in Aims 1 and 2. In the secondary analysis, we will model mean hypoglycemia awareness score by domain (e.g., autonomic, neuroglycopenic, and malaise).

6.5.3 Safety – N/A

6.5.4 **Analysis of Subject Characteristics:** Characteristics of the study population will be characterized using means (standard deviation, SD), and counts (%).

6.5.5 Interim Analysis (if applicable) – N/A

6.5.6 Health economic evaluation – N/A

6.5.7 Other - N/A

6.5.8 Subsets and Covariates - confounding variables include of age, BMI, and duration of diabetes. Details of the plan to evaluate confounding variables are above in Statistical Aim 1.

6.5.9 Handling of Missing Data – Participants that are unable to wear their CGM for at least 75% of the time will be excluded from the analysis.

7 Trial Administration

7.1 Ethical Considerations: Informed Consent/Assent and HIPAA Authorization: The study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. To help offset the time and cost for subjects' participation in the study, they will receive $120 for each of the HRU/CSRU/Masonicare visits (visits 3 and 5), including euglycemic-hypoglycemic clamp visit. Additionally, participants will be compensated $10 for visits 1, 2 and 4 and $50 per 2-week diet record that is completed at least 70% or 5/7 days per week. At the end of the study, each participant will be compensated $30 for wearing the CGM at least 85% of the time. Participants will receive $20 for completing the interview at Visit 6. Each participant will receive up to a total of $420 for completing all aspects of the study. Participants or their guardians will receive YNHH parking garage voucher for overnight visit at YNHH research unit visit. Value for parking up to $40 for 2-night stay (visit 3 and visit 5). Any sensitive data that is collected (pregnancy test result) will be discussed with the participant only to protect confidentiality. This will be documented as a sensitive note in the patient's chart. Study visits will be added to the patient's medical record to ensure other providers that participate in your care are aware of your participation in the study.

7.2 Institutional Review Board (IRB) Review: The protocol will be submitted to the IRB for review and approval. Approval of the protocol must be obtained before initiating any research activity. Any change to the protocol or study team will require an approved IRB amendment before implementation. The IRB will determine whether informed consent and HIPAA authorization are required. The IRB will conduct continuing review at intervals appropriate to the degree of risk, but not less than once per year. A study closure report will be submitted to the IRB after all research activities have been completed. Other study events (e.g. data breaches, protocol deviations) will be submitted per Yale University IRB's policies.

7.3 Subject Confidentiality: Subject confidentiality is held in strict trust by the research team. Participant medical record review will be limited to only the elements needed to complete the study. Only authorized HIPAA and GCP trained study team members will be allowed to extract research data from medical records and enter it into Microsoft Excel databases on university computers which are encrypted and served by a regular back up service. All study staff, office, and computers are HIPPA compliant. Data may also be collected using Qualtrics, which is also HIPAA compliant. No direct subject identifiers will be entered into the research database. Each subject will be assigned a unique study number. A master list linking the unique study number to the human subject will be maintained in a locked drawer at the pediatric diabetes research center, which is protected by badge access and locked doors. Only study staff listed on the protocol will have access to this information.

7.4 Deviations/Unanticipated Problems: If the study team becomes aware of an anticipated problem (e.g. data breach, protocol deviation), the event will be reported to the IRB by IRES.

7.5 Data Collection: Clinical research forms will be created to allow collection of data from participants. Some data will be collected using Qualtrics or RedCap which are both HIPAA compliant. Once collected, data will be de-identified and study records will be maintained in databases, on university computers which are encrypted and served by a regular back up service. All study staff, office, and computers are HIPPA compliant. Identifiers will be destroyed 24 months after study completion, however de-identified information will be kept indefinitely.

7.6 Data Quality Assurance: All investigators on the study have completed Good Clinical Practice Training and will continue to do so on a regular basis through the Yale Center for Clinical Investigation. Data quality will be ensured by the principal investigator, Laura Nally. All research staff will meet quarterly to ensure data collection is consistent and complete.

7.7 Study Records: Study records will include protocols, consent forms, surveys filled out by participants, and source documents.

7.8 Access to Source Documents: Source documents will only be available to those listed on the protocol. De-identified information will be used whenever possible. Only de-identified information will be transferred to collaborators.

7.9 Data or Specimen Storage/Security: Data will be collected on digital source documents and using Qualtrics and/or RedCap. Digital source documents will be stored on encrypted computers. Hard copies of consent forms will be kept in a locked file cabinet within the PI's office, in an area that requires badge access. De-identified data will be kept indefinitely. Blood samples collected as part of this research will be stored by the YCCI core lab services.

7.10 Retention of Records: Study documents will be retained for a 2 years, but de-identified information will be kept indefinitely. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

7.11 Study Monitoring: The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting safety reviews every 6 months (including when reapproval of the protocol is sought. During the review process, the principal investigator will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator or the IRB have the authority to stop or suspend the study or require modification.

7.12 Data Safety Monitoring Plan: Greater Than Minimal Risk DSMP

1. Personnel responsible for the safety review and its frequency:

The principal investigator will be responsible for monitoring the data, assuring protocol compliance, and conducting the safety reviews at the specified frequency, which must be conducted at a minimum of every 6 months (including when reapproval of the protocol is sought). During the review process, the principal investigator (monitor) will evaluate whether the study should continue unchanged, require modification/amendment, or close to enrollment. Either the principal investigator, the IRB or [*enter the names of other oversight bodies that have this authority, e.g.,* Yale Cancer Center Data and Safety Monitoring Committee (DSMC)] have the authority to stop or suspend the study or require modifications.

2. The risks associated with the current study are deemed greater than minimal for the following reasons: (choose those that apply)

1. We do not view the risks associated with the hyperinsulinemic euglycemic-hypoglycemic clamp as minimal risks.
2. Given the now established safety and validity of the current hyperinsulinemic clamp protocols in our prior work, we do not view the proposed studies as high risk.

Although we have assessed the proposed study as one of greater than minimal risk, the potential exists for anticipated and/or unanticipated adverse events, serious or otherwise, to occur since it is not possible to predict with certainty the absolute risk in any given individual or in advance of first-hand experience with the proposed study methods. Therefore, we provide a plan for monitoring the data and safety of the proposed study as follows:

3. Attribution of Adverse Events:
Adverse events will be monitored for each subject participating in the study and attributed to the study procedures / design by the principal investigator (*Laura Nally*) according to the following categories:

a.) Definite: Adverse event is clearly related to investigational procedures(s)/agent(s).
b.) Probable: Adverse event is likely related to investigational procedures(s)/agent(s).
c.) Possible: Adverse event may be related to investigational procedures(s)/agent(s).
d.) Unlikely: Adverse event is likely not to be related to the investigational procedures(s)/agent(s).
e.) Unrelated: Adverse event is clearly not related to investigational procedures(s)/agent(s).

4. Plan for Grading Adverse Events:

The following scale will be used in grading the severity of adverse events noted during the study:

1. Mild adverse event
2. Moderate adverse event
3. Severe

5. Plan for Determining Seriousness of Adverse Events:

Serious Adverse Events:
In addition to grading the adverse event, the PI will determine whether the adverse event meets the criteria for a Serious Adverse Event (SAE). An adverse event is considered serious if it results in any of the following outcomes:

1. Death;
2. A life-threatening experience in-patient hospitalization or prolongation of existing hospitalization;
3. A persistent or significant disability or incapacity;
4. A congenital anomaly or birth defect; OR
5. Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

An adverse event may be graded as severe but still not meet the criteria for a Serious Adverse Event. Similarly, an adverse event may be graded as moderate but still meet the criteria for an SAE. It is important for the PI to consider the grade of the event as well as its "seriousness" when determining whether reporting to the IRB is necessary.

6. Plan for reporting UPIRSOs (including Adverse Events) to the IRB

The principal investigator will report the following types of events to the IRB:

Any incident, experience or outcome that meets ALL 3 of the following criteria:

1. Is unexpected (in terms of nature, specificity, severity, or frequency) given (a) the research procedures described in the protocol-related documents, such as the IRB-approved protocol and informed consent document and (b) the characteristics of the subject population being studied; AND
2. Is related or possibly related to participation in the research (*possibly related* means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); AND
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, legal, or social harm) than was previously known or recognized.

Unanticipated Problems Involving Risks to Subjects or Others (UPIRSOs) may be medical or non-medical in nature, and include – but are not limited to – *serious, unexpected, and related adverse events* and *unanticipated adverse device effects*. ***Please note*** that adverse events are reportable to the IRB as UPIRSOs **only** if they meet all 3 criteria listed above.

These UPIRSOs/SAEs will be reported to the IRB in accordance with IRB Policy 710, using the appropriate forms found on the website. All related events involving risk but not meeting the *prompt* reporting requirements described in IRB Policy 710 should be reported to the IRB in summary form at the time of continuing review. If appropriate, such summary may be a simple brief statement that events have

occurred at the expected frequency and level of severity as previously documented**.** In lieu of a summary of external events, a current DSMB report can be submitted for research studies that are subject to oversight by a DSMB (or other monitoring entity that is monitoring the study on behalf of an industry sponsor).

7. Plan for reporting adverse events to co-investigators on the study, as appropriate the protocol's research monitor(s), e.g., industrial sponsor, Yale Cancer Center Data and Safety Monitoring Committee (DSMC), Protocol Review Committee (PRC), DSMBs, study sponsors, funding and regulatory agencies, and regulatory and decision-making bodies.

For the current study, the following individuals, funding, and/or regulatory agencies will be notified (choose those that apply):

X All Co-Investigators listed on the protocol.

☐ Yale Cancer Center Data and Safety Monitoring Committee (DSMC)

X National Institutes of Health

☐ Food and Drug Administration (Physician-Sponsored IND #_____)

☐ Medical Research Foundation (Grant_____)

☐ Study Sponsor

☐ Other Data Safety Monitoring Board (DSMB) or Committee (DSMC)

The principal investigator (*Laura Nally*) will conduct a review of all adverse events upon completion of every study subject. The principal investigator will evaluate the frequency and severity of the adverse events and determine if modifications to the protocol or consent form are required.

Please note: For any study that may be considered high risk, the IRB will be more focused on the safety requirements for the study and a DSMB will likely be required.

7.13 Study Modification: If a study modification is deemed necessary, it will be submitted through IRES to the IRB. Any protocol changes will not be implemented until they are approved by the IRB.

7.14 Study Discontinuation: If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

7.15 Study Completion

- The study will be completed when all participants have completed all study interventions.

7.16 Conflict of Interest Policy: The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the appropriate conflict of interest review committee has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest. All investigators will follow the applicable conflict of interest policies.

7.17 Funding Source: This study is funded by the Diabetes Research Center DRC (P30 DK045735) and NIH/NIDDK K23 DK128560-01.

7.18 Publication Plan: It is likely that data from this study will be published in scientific and medical journals and presented at scientific and medical conferences. In all such cases, project data will be presented in such a way that no participant could possibly be identified. Dr. Nally is primarily responsible for the study results.

8 Appendices

Appendix #	Title	Section	Topic
Appendix 1	**Stable isotopes**		**Information about stable isotopes**

Appendix 1

Stable Isotopes: All substances are non-radioactive and have been extensively used in pediatric research in the last 15 years by our team with no side effects or adverse effects. Recent studies involving minors using glucose and glycerol isotopes performed by members of our research team are listed below. Isotopes will be obtained from Cambridge Isotopes, Cambridge, MA), which is routinely audited internally by its QA department and externally by customers, notified bodies, and regulatory agencies (e.g. FDA). Isotopes will be stored in the Investigational Pharmacy and will be prepared before the subjects undergo tests.

HIC #	Age group	Isotope	Dosage	Isotope	Dosage
0102012241	8 years-adult	6,6-D_2-glucose*	0.4 g/m^2 bolus	D_5-glycerol	0.6 μmol/m^2 4 μmol/m^2·min
1604017607	12-30 years	6,6D_2-glucose*	0.4 g/m^2 bolus	D_5-glycerol	0.6 μmol/m^2 4 μmol/m^2·min
1306012200	≥ 12 to ≤ 20 yrs Type 1 Diabetes	6,6-$^2H_2$-glucose	4.5 mg/kg bolus 0.03 mg/kg/min	$^2H_5$-glycerol	1.6μmol/kg, 0.11 μmol /kg/min
1509016531	12-35 years Type 1 Diabetes	6,6-$^2H_2$-glucose	3.5 mg/kg bolus 0.04 mg kg/min		
2000023149	12-16 years 18-24 years	6,6-$^2H_2$-glucose	4.5 mg/kg bolus 0.06 mg/kg/min	$^2H_5$-glycerol	1.6μmol/kg, 0.11 μmol /kg/min

*Stable phases will use 20% glucose infusion containing 6,6,-D2-glucose at an enrichment of approximately 3%

PMID	Age group	Isotope	Dosage	Isotope	Dosage
30020457	14-17 years Type 1 Diabetes	6,6-$^2H_2$-glucose	4.5 mg/kg 0.03 mg/kg/min	$^2H_5$-glycerol	1.6μmol/kg, 0.11μmol /kg/min

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Table 6. Blood volume collected during each HRU/CSRU/Masonicare visit.

Weight (kg) at Blood Draw	Total Blood Volume Collected (mL)	Total Blood Volume Collected (mL/kg)
43	208.7	4.85
50-60	208.7	4.17-3.47
>60	208.7	<3.47