HIC# 2000023149

HRP-503B – BIOMEDICAL RESEARCH PROTOCOL
(2017-1)

Protocol Title: Effect of Adiposity on Hepatic and Peripheral Insulin Resistance in Type 1 Diabetes

Principal Investigator: Michelle Van Name, MD

Version 4 Date: 8-31-18

(*If applicable*) Clinicaltrials.gov Registration #: NCT2000023149

INSTRUCTIONS
This template is intended to help investigators prepare a protocol that includes all of the necessary information needed by the IRB to determine whether a study meets approval criteria. **Read the following instructions before proceeding:** 1. Use this protocol template for a PI initiated study that includes direct interactions with research subjects. Additional templates for other types of research protocols are available in the system Library. 2. If a section or question does not apply to your research study, type "Not Applicable" underneath. 3. Once completed, upload your protocol in the "Basic Information" screen in IRES IRB system.

APPROVED BY THE YALE UNIVERSITY IRB 9/14/2018 VALID THROUGH 8/21/2019

SECTION I: RESEARCH PLAN

1. **Statement of Purpose:** State the scientific aim(s) of the study, or the hypotheses to be tested.
   -To assess the effects of adiposity on resistance to insulin's ability to suppress hepatic glucose production and to stimulate peripheral glucose metabolism in adolescents with type 1 diabetes
   -To examine the role of fatty liver disease on the insulin resistance of obesity in adolescents with type 1 diabetes

2. **Probable Duration of Project:** State the expected duration of the project, including all follow-up and data analysis activities.
   4 years

3. **Background:** Describe the background information that led to the plan for this project. Provide references to support the expectation of obtaining useful scientific data.

Obesity in youth with type 1 diabetes (T1D) is an epidemic that developed after the implementation of intensive insulin management in the 1980s; an issue that was identified and highlighted by the weight gain seen in the DCCT intensive treatment group (1). While it has long been recognized that the peripheral insulin resistance associated with puberty in lean adolescents is a substantial obstacle to optimal metabolic control in adolescents with T1D (2), excessive weight gain is now recognized as an additional challenge in the management of youth with T1D. However, the effect of obesity on peripheral and hepatic insulin sensitivity in pubertal adolescents with T1D has not been well studied.

Many adolescents with T1D struggle with a vicious cycle in which the insulin resistances of obesity and puberty necessitate ever higher doses of insulin, which contribute to additional weight gain that further exaggerates insulin resistance. This is illustrated by the large proportion of adolescents and young adults in the T1D Exchange (T1DX) Registry who are unable to achieve and maintain HbA1c levels in the target range (3). When compared to participants with well controlled diabetes in the T1DX Registry, adolescents and young adults with poorly controlled T1D were nearly two-fold more likely to have BMI >85% (4), however, the characteristics of this obesity/puberty phenotype are unknown. For example, obesity in adolescents with T1D may lead to severe hepatic, as well as peripheral insulin resistance, which contributes to post-meal hyperglycemia and increased HbA1c levels due to inability of pre-meal boluses to suppress endogenous glucose production.

The rate of overweight and obesity in patients with T1D mirror that seen in the general population nationwide (5), with recent data from the T1DX revealing that 40% or more adolescents and young adults are overweight or obese (3). Our experience in the Yale Pediatric Diabetes Program reflects similar rates of this new phenotype, and we may expect further increases in the prevalence of obesity in T1D as clinicians attempt to optimize metabolic control with insulin alone. The much anticipated availability of the first commercial hybrid closed loop (HCL) insulin delivery system has been shown to significantly improve diabetes management in a multitude of ways: lower HbA1c, increased time in target sensor glucose range, less frequent hypoglycemia (6), and decreased diabetes burden (7). However, the improved glycemic control seen in adolescents was accompanied by an 8% increase in total daily insulin dose, as well as 1 kg weight gain during three months of study. While the increase in weight in adolescents in this study did not reach statistical significance, the findings in the much larger group of adults of a 1.4 kg weight increase weight in three months was statistically significant (6). Increases in weight are not surprising, as increases in daily insulin doses are associated with weight gain (8), which is mediated in part by reductions in renal glucose excretion. [redacted] s we prepare to initiate this study, it is evident that use of this and other hybrid closed loop systems will dramatically rise during the timeline of this three-year study,

which will lead to improved glycemic control and higher insulin doses that fuel further increases in severity and rates of obesity in youth with T1D.

While new drugs have become available that hold promise as adjunctive agents to improve glycemic control, lower insulin doses, and promote weight loss, their impact on glucose and fat metabolism in muscle and adipose tissue in adolescents in the setting of obesity and T1D is unknown. Identifying the areas of significant insulin resistance in obese youth with T1D must be elucidated prior to identifying and testing therapies to improve glycemic control and weight.

Decreasing the risk of microvascular and macrovascular complications has been a main driving factor in improving management of T1D in youth, with the effects of intensive management providing protection from progression to atherosclerosis and cardiovascular disease. However, recent evidence indicates that the insulin resistance resulting from the weight gain that accompanies intensive management may counter some of the benefits gained from improved glycemic control on CVD risk (9,10). Nevertheless, as noted previously, the effects of obesity on metabolic control and insulin sensitivity in youth with T1D have yet to be rigorously studied.

Hepatic and peripheral insulin sensitivity in youth with T1D

The hyperinsulinemic-euglycemic clamp technique (17) was first adapted for use in children and adolescents by Dr. Tamborlane (my primary mentor) and his colleagues in a study that demonstrated that the hormonal changes of puberty induced a physiologic state of insulin resistance in healthy lean adolescents compared to healthy lean preadolescents and young adults (2). In follow-up studies, these investigators used the stepped hyperinsulinemic-euglycemic clamp in combination with stable isotope infusions to demonstrate that the insulin resistance of normal puberty was related to impairments in insulin's ability to stimulate peripheral glucose uptake; whereas, insulin's ability to suppress hepatic glucose production did not appear to be adversely affected (11). Dr. Caprio (my secondary mentor) has used similar techniques to describe, in depth, the metabolic consequences of obesity in non-diabetic children over the past 20 years (12-20), including indirect calorimetry to measure rates of glucose and fat oxidation. Nevertheless, the effect of obesity on hepatic and peripheral insulin sensitivity in adolescents with T1D during puberty has not been established; this is the gap in knowledge that AIM 1 of this study is intended to fill. This Aim is particularly significant, as adolescents with T1D have the poorest glycemic control and may gain the most benefit from pharmacologic intervention to improve insulin sensitivity, promote weight loss, and reduce cardiovascular complications.

Based on recent work establishing branched chain amino acids (BCAAs) and α-hydroxybutyrate as markers of peripheral and hepatic insulin resistance in obese youth without T1D (21,22), I will work [redacted] to use targeted nuclear magnetic resonance (NMR) spectroscopy to assess these relationships in lean and obese adolescents with T1D. Validation of these recently identified biomarkers reflecting peripheral or hepatic insulin resistance in adolescents in our study cohorts will help identify patients who may particularly benefit from targeted early interventions.

Fatty liver disease in obese adolescents with T1D

An emerging body of evidence indicates that fatty liver disease, as assessed by abdominal Magnetic Resonance Imaging (MRI), has adverse effects on glucose metabolism, especially in adults with T2D (23). Some but not all studies have reported that non-alcoholic fatty liver disease is common in adults with T1D (24-26) and presence of this co-morbidity is associated with distal neuropathy (27) cardiovascular disease (24) and drastically increases the risk of cardiovascular events (hazard ratio 8.16) (25). It is particularly noteworthy that hepatic fat content in children and adolescents with T1D has only been studied using gold standard MRI techniques by Regnell and colleagues (28). In that study, hepatic fat content was slightly lower in 22 T1D subjects (1.3%) than in 32 controls without diabetes (1.8%), however, that study did not evaluate the effects of obesity on hepatic fat.

In contrast to the increased rates of lipolysis associated with loss of insulin sensitivity, physiologic mechanisms have been proposed to promote hepatic lipid accumulation in T1D. Abnormal lipoprotein function or ratio may limit triglyceride transport out of the liver via VLDL (29). Furthermore, transcription factors of hepatic lipogenesis (ChREBP and SREBP-1c) are stimulated under hyperglycemic conditions (29).

Dr. Caprio has extensive experience in the use of MRI to assess hepatic fat content in obese youth without diabetes (12,30,31). Her prior work in adolescents with obesity has shown that high liver fat content is associated with significant increases in insulin resistance. More specifically, high hepatic fat fraction is associated with an impaired action of insulin in both the liver and muscle, and may also limit glycerol turnover (12). I will use this technology to examine the putative role of fatty liver disease (Aim 2) on the development of hepatic insulin resistance of obesity in adolescents with T1D studied in AIM 1.

Innovation

A major focus of this program of research will be directed at advancing the understanding of the metabolic consequences of obesity and puberty in adolescents with T1D. For many years, our center has been at the forefront of studies that combined euglycemic-hyperinsulinemic clamps with non-radioactive stable isotope infusions to describe the insulin resistance of puberty in youth with and without T1D and the insulin resistance of obesity in non-diabetic children [1-5]. *Thus, an innovative aspect of Aim 1 is that it will be the first to use these sophisticated metabolic techniques to examine the effects of obesity and hepatic steatosis on insulin sensitivity in pubertal adolescents with T1D; namely, the 2-step hyperinsulinemic euglycemic clamp with tracer enhancement, which will allow for definition of hepatic and peripheral insulin resistance, glycerol turnover, and glucose and fat oxidation (Aim 1). A second novel aspect is that this will be the first study to utilize gold standard MRI methods to quantify and compare intrahepatic fat content in lean and obese adolescents with T1D (Aim 2). This will allow a global and more detailed understanding of the potential alterations of insulin's effects on key insulin sensitive tissues in youth that are impacted by both T1D and obesity.* Furthermore, evaluation of biomarkers for insulin resistance and fatty liver disease in this population will be performed for the first time.

Understanding the metabolic phenotype and the prevalence of nonalcoholic hepatic steatosis in overweight/obese adolescents with T1D are the critical first steps towards designing therapeutic interventions to improve metabolic control in these youth and to improve their long term cardiovascular outcomes. The knowledge gained from this program of research will inform selection of adjunctive pharmacologic agents to target insulin resistance in specific tissues. For example, adolescents with significant hepatic steatosis and hepatic insulin resistance may benefit from treatment with SGLT2i therapy given its potential to independently improve hepatic steatosis, beyond the weight loss effects. My future work will focus on the ability of adjunctive pharmacologic agents to impact turnover of lipid and glucose, as well as reduce hepatic and peripheral insulin resistance and hepatic fat, which are currently unexplored in adolescents with T1D, as initial studies in this area focused on safety and glycemic outcomes.

I anticipate that the results of this proposal will help to inform future studies of SGLT2 inhibitors in pediatric patients with T1D. Moreover, identification of markers associated with insulin resistance and progression of fatty liver disease [6] will facilitate identification of participants who stand to benefit from treatment in future studies.

Future projects to understand and the scientific premise, rationale, and mechanisms of adjunctive therapies in T1D

In broad terms, treatment of T1D has not changed in almost 100 years; simply more and better types of insulin delivered in different ways. Thus far, the only adjunctive agent studied in youth with T1D is metformin, but the benefits of treatment with this agent were disappointing. Yale was one of the centers

in the recent randomized clinical trial of metformin carried out by the T1D Exchange (T1DX) Study Group in overweight and obese adolescents with T1D. That study showed that adjunctive treatment with metformin did not result in improved glycemic control, although there were small reductions in insulin dose and BMI z-scores (32). The T1DX metformin study was the first and only study to date aimed at treating this new phenotype of youth with obesity and T1D. Furthermore, our group has found only modest improvements in postprandial glycemic excursions with use of a GLP-1 agonist in adults with T1D (33).

There are a number of reasons why SGLT2 inhibitors are particularly attractive agents for adjunctive treatment of overweight and obese individuals with T1D: several drugs in this class have been approved for use in adults with T2D; their mechanism of action does not depend on stimulation of endogenous insulin secretion; treatment with these agents lowers weight, blood pressure, and HbA1c; and recent studies indicate that these agents have cardio- and reno-protective benefits (34). Sophisticated metabolic studies in adults with T2D have shown improvements in insulin sensitivity (35,36), likely due to decreasing glucotoxicity; results that should be translatable to T1D. In fact, improved HbA1c, fasting glucose, increased time in range, as well as decreased insulin requirements and weight loss, have been seen when SGLT2i are used as adjunctive therapies in adults with T1D (37-39). Moreover, use of an oral medication may be less burdensome than additional injectables for future use in youth with T1D who already struggle with current diabetes management tasks. Investigators have demonstrated that two to four weeks of treatment with SGLT2i results in a shift from glucose oxidation to fat oxidation (35,36) in adults with T2D. Additionally, a small study has shown that SGLT2i therapy lowers ALT in adults with T2D and ultrasound diagnosed hepatic steatosis, unrelated to weight loss (40). [redacted]

4. **Research Plan:** Summarize the study design and research procedures using non-technical language that can be readily understood by someone outside the discipline. **Be sure to distinguish between standard of care vs. research procedures when applicable, and include any flowcharts of visits specifying their individual times and lengths.** Describe the setting in which the research will take place.

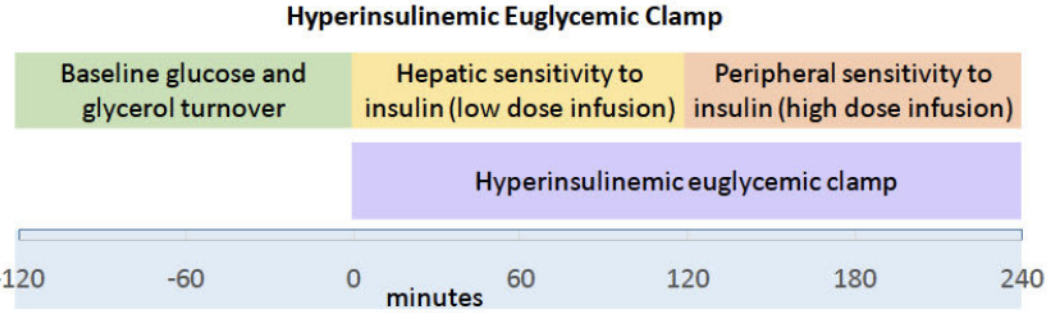
   Overview: This is a cross-sectional study of 72 adolescents and 36 young adults with T1D who will undergo comprehensive metabolic evaluation. The study consists of 2 visits which will take place over approximately 30 days. The study aims to enroll 36 adolescents with T1D and overweight/obesity, 36 lean adolescents with T1D, and 36 young adults with T1D. To characterize the impact of adiposity on metabolism during puberty, adolescents will undergo the euglycemic hyperinsulinemic clamp study with tracer enhancement and DEXA scan on the Hospital Research Unit, and abdominal MRI in the Magnetic Resonance Resource Center. The lean young adults with T1D will provide a control group to reflect prepubertal youth with T1D, and will undergo the euglycemic hyperinsulinemic clamp study with tracer enhancement and DEXA scan.

Subjects: This study will enroll 36 obese/overweight and 36 lean adolescents (Tanner stage 2-5) with T1D. A comparison control group of 36 lean young adults with T1D will also be enrolled, since they will be unaffected by the adverse metabolic effects of puberty or obesity.

Study visits: Enrollment and both study visits will take place over approximately 30 days. All participants will undergo screening to evaluate study eligibility through the elicitation of a medical history and physical examination and laboratory tests.

Enrollment: Screening will be conducted by invitation to potentially eligible subjects during which written informed consent will be obtained prior to conducting any study related procedures. After 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 30 days may be used.

Visit 1: A hyperinsulinemic-euglycemic clamp will be conducted as a gold standard assessment of insulin sensitivity, with infusion of a stable glucose isotope in order to distinguish hepatic from peripheral insulin action.



Clamp studies will be carried out following methods utilized in Dr. Caprio's lab [3,5], as described briefly below. Participants will be admitted to the Hospital Research Unit (HRU) the evening prior to the clamp procedures for transition to IV insulin and stabilization of blood glucose with target of 90-100. On admission urine will be dipped for ketones, and if present, ketones will be managed as per routine clinical practice. If present, ketones will be monitored throughout treatment and the study will be delayed or cancelled pending resolution of ketosis. All participants will be advised to limit exercise in the 3 days prior to study to limit acute effects of recent exercise on insulin sensitivity. Females in the study who have reached menarche and have regular menses will be asked to keep a menstrual diary so that we can plan to study them during the follicular phase of the menstrual cycle (more insulin sensitive phase).

Baseline assessments will include liver function tests, lipids, sex steroids, IGF-1 (measure of growth hormone secretion), isoleucine, leucine, valine, and α-hydroxybutyrate.

- *Non-radioactive, Stable Isotope Tracer Infusions* will be employed to assess rates of glucose and lipid metabolism at the end of the 120 min baseline equilibration period and during the two stepped IV insulin infusion periods. From -120 to 0 min, 4.5 mg/kg of 6,6-$^2H_2$-glucose (99% enriched; Cambridge Isotope Laboratories, Andover MA) will be followed by a continuous infusion at 0.03 mg/kg/min[7]. $^2H_5$-glycerol will be infused at 1.6 μmol/kg followed by a constant infusion of 0.11 μmol/kg/min[7] to assess glycerol turnover. During the 120 min basal turnover period, a variable rate insulin infusion will be utilized to maintain plasma glucose between 90-110 mg/dL.
- *Low Dose Insulin Infusion Period to Assess Hepatic Sensitivity to Insulin:* After obtaining baseline samples, subjects will receive a 10 mU/M^2 surface area /min infusion of insulin infusion for 120 minutes along with a variable 20% dextrose infusion with a goal of maintaining plasma glucose at 90-95 mg/dL.
- *High Dose Insulin Infusion Period to Assess Peripheral Sensitivity to Insulin:* The high dose insulin infusion of 80 mU/M^2 surface area/min will also continue for 120 minutes accompanied by a

variable 20% dextrose infusion [5]. 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.

- *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 final 30 minutes of the baseline equilibration phase, as well as during the final 30 minutes of the low and high dose insulin infusion phases.
- *Measures of Oxidation:* Indirect calorimetry will be utilized at baseline and the end of each step of the clamp to evaluate rates of glucose and fat oxidation.

DEXA scan (Hologic, Boston, MA, USA): Total body fat and lean body mass will be assessed by dual energy x-ray absorptiometry (DEXA).

Visit 2: Adolescent participants (age 12-16). Young adults will not participate in this visit. Participants with transaminases >2.5 the upper limit of normal will undergo clinical evaluation to exclude liver disease (other than non-alcoholic steatosis) prior to proceeding with MRI.

Participants will present to the Magnetic Resonance Research Center (MRRC) for abdominal MRI. Using a 3Tesla Magnet (Siemens), we will assess intra-abdominal fat deposition and analyze the subcutaneous and visceral using methods our group has employed in the past [8].

We will quantitate the liver fat accumulation using the magnitude-based MRI of the liver to determine MRI-estimated Proton density fat fraction (PDFF) [9]. The PDFF strongly correlates with hepatic steatosis as assessed by histopathology of liver biopsy. The 3T magnet will be used to scan participants and the gradient-recalled-echo technique will estimate liver PDFF [9].

5. Genetic Testing N/A ☒
   - **A.** Describe
     - i. the types of future research to be conducted using the materials, specifying if immortalization of cell lines, whole exome or genome sequencing, genome wide association studies, or animal studies are planned *Write here*
     - ii. the plan for the collection of material or the conditions under which material will be received *Write here*
     - iii. the types of information about the donor/individual contributors that will be entered into a database *Write here*
     - iv. the methods to uphold confidentiality *Write here*
   - B. What are the conditions or procedures for sharing of materials and/or distributing for future research projects? *Write here*
   - C. Is widespread sharing of materials planned? *Write here*
   - D. When and under what conditions will materials be stripped of all identifiers? *Write here*
   - E. Can donor-subjects withdraw their materials at any time, and/or withdraw the identifiers that connect them to their materials? *Write here*
     - i. How will requests to withdraw materials be handled (e.g., material no longer identified: that is, anonymized) or material destroyed)? *Write here*
   - F. Describe the provisions for protection of participant privacy *Write here*
   - G. Describe the methods for the security of storage and sharing of materials *Write here*

6. **Subject Population:** Provide a detailed description of the types of human subjects who will be recruited into this study.
   This study aims to enroll 36 obese/overweight adolescents and 36 lean adolescents with T1D. A comparison control group of 36 lean young adults with T1D matched for gender and HbA1c will also be enrolled, since they will be unaffected by the adverse metabolic effects of puberty or obesity.

7. **Subject classification:** Check off all classifications of subjects that will be specifically recruited for enrollment in the research project. Will subjects who may require additional safeguards or other considerations be enrolled in the study? If so, identify the population of subjects requiring special safeguards and provide a justification for their involvement.

☒Children	☐ Healthy	☐Fetal material, placenta, or dead fetus
☐Non-English Speaking	☐ Prisoners	☐Economically disadvantaged persons
☐Decisionally Impaired	☐ Employees	☐Pregnant women and/or fetuses
☐Yale Students	☒ Females of childbearing potential	

NOTE: Is this research proposal designed to enroll children who are wards of the state as potential subjects?
Yes ☐ No ☒

To understand the effects of adiposity in puberty on metabolism in type 1 diabetes, we must study adolescents during puberty, and this must also occur in a pediatric population as adolescents usually complete puberty by age 18. Furthermore, this age group contains females of childbearing potential and exclusion of females would limit the findings only to males. Our group is experienced in conducting euglycemic hyperinsulinemic clamps and abdominal MRI in children. Prior published work is evidence of our scientifically rigorous process while maintaining safety for study participants.

8. **Inclusion/Exclusion Criteria:** What are the criteria used to determine subject inclusion or exclusion?

• Inclusion criteria:

All Participants:

1. Clinical diagnosis of T1D
2. HbA1c ≤9%
3. Diabetes duration of at least 12 months

Adolescents with T1D:

1. Age 12-16 years
2. BMI <75th for lean pediatric subjects, ≥ 85th percentile for overweight/obese pediatric subjects;
3. Tanner stage 2-5
4. Parent able to provide written consent and participant able to provide assent

Lean, young adults with T1D:

1. Age 18-24 years
2. BMI 18.5-24.9 kg/m2
3. Able to provide written consent.

• Exclusion criteria:

1. Use of adjunctive diabetes medications or other medications known to impact insulin sensitivity
2. Weight loss medications within the past six months
3. Current psychiatric disorders, including eating disorders (DSM-V criteria)

4. Known liver disease other than nonalcoholic hepatic steatosis
5. Females who are pregnant or lactating
6. Anemia or another medical condition that precludes participation in the study
7. Not meeting MRI safety criteria
8. Claustrophobia that will prevent participation in the MRI

9. How will **eligibility** be determined, and by whom? Write here
   PI or co-investigators will determine eligibility after review of medical history, screening tests and inclusion/exclusion criteria through the regular clinical encounters and through review of the EMR record of patients under their clinical care

10. **Risks:** Describe the reasonably foreseeable risks, including risks to subject privacy, discomforts, or inconveniences associated with subjects participating in the research.

The risk of disclosure of protected health information is small. Efforts are taken and procedures are in place to assure that this does not occur, in compliance with HIPAA/HITECH/future privacy regulations.

i. Risks related to the hyperinsulinemic euglycemic clamp:

(a) The blood draws and IV placement could result in discomfort or bruising, or rarely and infection or blood clot

(b) Hypoglycemia/Hyperglycemia:. During these clamps insulin is infused, which can cause hypoglycemia. For this reason, blood glucose is measured frequently throughout the study by a reliable device (YSI 2300) so that the rate of dextrose infusion can be adjusted regularly to maintain the blood glucose at target and prevent hypoglycemia. The study procedures may result in elevated or low glucose values. At the end of each visit, the participant will receive guidance for glucose monitoring, food intake, and insulin dosing as needed.

Furthermore, our group is very experienced in conducting these studies. Additionally, when we began doing these studies, there was concern of adverse psychological effects on children which we carefully assessed in early studies. Dr. Caprio found no adverse impact of clamp studies on children.

(c) Stable isotopes: Despite the theoretical risk of infection with infusion of stable isotopes, our team has been using isotopes during clamp studies for 15 years in children and adolescents and have not experienced any adverse events.

(d) Risk of indwelling catheter: There are potential complications associated with indwelling catheters, including hematomas, discomfort, and rarely infection, thrombophlebitis, or bleeding at the catheter insertion site.

(e) Anemia: The risk of anemia exists in studies with frequent blood draws. In our many years of performing clamp studies we have not known any participants to experience this complication.

ii. Risks related to imaging assessments:

(a) DEXA Scan: There is radiation exposure during the DEXA Scan, which is estimated to be less than 1/10 the radiation individuals receive during a chest X-ray. It is below the FDA limits for radiation exposure in research subjects. We have routinely used DEXA to monitor body composition in children.

(b) Abdominal MRI: MRI uses magnetism and radio waves to create images of various body parts. MRI does not use x-rays to create images. Any metal in the scanner room is dangerous due to the strong magnets in the scanner, which can attract metal. The scan should take no more than 45 minutes.

11. **Minimizing Risks:** Describe the manner in which the above-mentioned risks will be minimized.

In order to protect the confidentiality of each participant, they will be assigned a coded identifier that will be used instead of their name.

i. Protection against risks related to the hyperinsulinemic euglycemic clamp:
(a) IV insertion will be performed by trained research nurses on the HRU. Numbing cream can be used to reduce the pain.

(b) Hypoglycemia/Hyperglycemia during clamp studies: Frequent blood glucose checks using a bedside analyzer will allow for careful monitoring so that the study doctors and nurses can identify trends that may lead to hypo or hyperglycemia. Infusion of both insulin and glucose allow for rapid correction of either situation. The study will be performed under the control of a physician.

(c) Stable isotopes: Isotopes will be carefully monitored and administered, and will be prepared in the investigational pharmacy to ensure proper technique.

(d) Risk of indwelling catheter: 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.

(e) Anemia: Participants will be screened with a hematocrit level prior to initiation of study procedures. 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 5cc/kg in pediatric participants and 550 ml in adult participants.

ii. Protection against risks related to imaging assessments:

(a) DEXA Scan: Subjects receive minimal radiation from a DEXA scan (0.01-0.04 millirem per measurement), which is estimated to be less than 1/10 the radiation that is received during a chest X-ray. These are below the FDA radiation exposure limits for research subjects. We carefully follow the United States Food and Drug Administration guidelines for magnet strength and radio wave exposure. Subjects will be asked about prior radiation exposure to ensure that cumulative annual exposure doesn't exceed FDA limits. All scans will be under medical supervision with trained nursing staff.

(b) MRI Scanning: We observe the FDA guidelines regarding magnet strength and radio wave exposure. Participants will be screened for magnetic resonance (MR) safety using the MR Safety Questionnaire, and we will encourage them to provide any information they think might be important. Participants and study staff involved in the scan will enter a detector designed to detect metal objects to minimize the possibility of metal entering the MR room. No metal can be brought in the MR room at any time. The door will be closed so that no individuals from outside accidentally enter the magnetic space. No person should have an MRI (Magnetic Resonance Imaging) test for any reason while either using a continuous glucose monitor (CGM) device normally or if there is possibly a broken sensor under the child's skin. Insulin pumps must be removed prior to MRI.

Participants will be closely monitored while in the scanner, and if they feel anxious or uncomfortable they will have the option to stop the study and come out of the MR scanner. Rarely, individuals in a MR scanner may experience dizziness, stomach upset, metallic taste in the mouth, tingling sensations or muscle twitching, and these experiences usually resolve quickly. Participants will be asked to let the research team know if they experience these sensations.

The images obtained are for research purposes and not for clinical diagnoses. If a concern arises based on a scan, we will ask a radiologist or other appropriate physician to review the finding. Based on this recommendation, if additional evaluation is needed, the PI will contact the patient or family to recommend they seek medical evaluation as a precautionary measure. The images obtained as part of this study are not for diagnostic purposes.

12. **Data and Safety Monitoring Plan:** Include an appropriate Data and Safety Monitoring Plan (DSMP) based on the investigator's risk assessment stated below. (Note: the HIC will make the final determination of the risk to subjects.)
    a. What is the investigator's assessment of the overall risk level for subjects participating in this study? Greater than minimal risk
    b. If children are involved, what is the investigator's assessment of the overall risk level for the children participating in this study? Greater than minimal risk
    c. Include an appropriate Data and Safety Monitoring Plan.

Greater Than Minimal Risk DSMP

This study is deemed greater than minimal risk with no prospect of direct benefit to the individual subjects involved in the research, but likely to yield generalized knowledge about the subject's disorder or condition

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 or the IRB 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 euglycemic hyperinsulinemic clamp as minimal risks.
2. Given the now established safety and validity of the current clamp and MRI procedures 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 Michelle Van Name 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 Michelle Van Name 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.

d. For multi-site studies for which the Yale PI serves as the lead investigator: N/A
   i. How will adverse events and unanticipated problems involving risks to subjects or others be reported, reviewed and managed? *Write here*
   ii. What provisions are in place for management of interim results? *Write here*
   iii. What will the multi-site process be for protocol modifications? *Write here*

13. **Statistical Considerations:** Describe the statistical analyses that support the study design.

Visit 1 Euglycemic Hyperinsulinemic Clamp: Sample Size: Prior work evaluating insulin sensitivity in youth with T1D is limited in consistency in reporting insulin-mediated glucose disposal. Thus, for sample size calculation, glucose infusion rate will be utilized as this calculation is more consistent across studies and can provide a good estimate of glucose disposal. A clinically meaningful difference would be at least 20% reduction in mean glucose disposal rate from lean to obese. In addition, we expect T1D lean young adults to have the highest mean glucose infusion rate, reflecting the anticipated response in T1D children not affected by hormonal changes of puberty (2). Therefore, we propose to compare the three means in a sequential manner: obese vs. lean adolescents with T1D, and lean adolescents with T1D vs. lean young adults with T1D, thus reducing the alpha level of 0.05 by ½.

Using published literature (43,44), we will need 36 lean and 36 obese/overweight pubertal adolescents with T1D, to have 80% at alpha of 0.025, to detect a difference between group means of 13.6 (SD=3) and 11.5 mg/kg lean body mass/min (SD=3); and 36 lean young adults to detect a minimum percent increase in glucose infusion rate in the range of 15-18% from that of lean pubertal adolescents with T1D - for the respective sequential group comparisons. Of note, the literature suggests that the actual increase for the latter is around 30% [2], therefore, we should be sufficiently powered.

An interim analysis will be conducted when we recruit 15 subjects in each group. If significant differences will be observed at that time, we will reconsider the sample size.

Data Analysis Approach: The departmental biostatistician Dr. Shabanova, will guide Dr. Van Name in analysis.

• Measures of Insulin Sensitivity: Calculations will be conducted using methods used by Drs. Caprio and Tamborlane in previous work (41). Endogenous glucose production and fasting insulin levels will be used to calculate hepatic insulin resistance. Glycerol turnover will be utilized to assess adipose specific insulin sensitivity.

• Statistical Analysis: Descriptive statistics, such as mean, standard deviation, count, and percent, will be used to characterize the groups. Continuous variables will be compared among the three groups using the Kruskal-Wallis test or Analysis of Variance (ANOVA) for not Normal and Gaussian distributions respectively, followed by pair-wise group comparisons with either the Student's t-test or the Mann-Whitney U test, depending upon the normality in the distributional assumptions of the variables. Categorical variables will be compared with the Chi-square test of the Fisher's Exact test. Spearman Correlation will explore associations of the following assessments with measures of insulin suppression of hepatic glucose production and stimulation of peripheral glucose uptake.

• Glycemic control (HbA1c, fasting glucose)

• Additional measures of insulin sensitivity (endogenous glucose production, glycerol turnover, glucose oxidation, fat oxidation).

Associations of these assessments with measures of insulin sensitivity will be explored for all groups. Unadjusted differences in the group means of primary and secondary outcomes of interest between lean and obese adolescent subjects, as well as between lean young adults and lean adolescent subjects, will be examined using the Student's t-test or the Mann-Whitney U test for variables with Gaussian and not normal distributions respectively. The impact of group, gender, sex hormones, pubertal stage, and growth factors on the outcome measures will also be explored using multivariable linear regression modeling. For the primary adjusted analysis, we will treat group as a categorical variable, and conduct two post-hoc pair-wise comparisons of means at alpha of 0.025. For the secondary adjusted analysis, we will also treat our three groups of subjects as an ordinal variable, whereby the lowest level will be given to the obese adolescents, followed by the lean adolescents, and then lean young adults. The latter approach will allow us to obtain a linear increase in the mean glucose disposal rate across the three groups, thus allowing us to use an alpha level of 0.05. The outcome variable will be normalized, if needed, using the natural log function.

Visit 2: Abdominal MRI: Sample Size: Based on mean hepatic lipid content in healthy overweight/obese and lean children [10], groups sizes of 36 lean and 36 obese adolescents will achieve >99% power at alpha of 0.05 to detect a difference between group means of 3.5% (SD=2) and 1.5% (SD=0.5). Even if a few subjects decline participation in this Aim, we will still have 90% power at alpha of 0.05 to detect the projected effect size using with a minimum of 12 subjects per group.

Data Analysis Approach:

The Proton Density Fat Fraction (PDFF) , a biomarker for hepatic steatosis, will be calculated from the liver images obtained liver PDFF parametric maps [9]. A PDFF value above 5.5% is indicative of fatty liver [11].

• *Statistical Analysis:* Descriptive statistics (mean, standard deviation, count, and percent) will be used to characterize the groups. Continuous variables will be compared between the lean and obese adolescents using either the Student's t-test or the Mann-Whitney U test. Categorical variables will be compared with the Chi-square test or the Fisher's Exact test. Associations of the following assessments with hepatic fat fraction will be explored through Spearman Correlation.

- Body composition (fat %, fat mass, lean mass)
- Abdominal fat distribution (visceral and subcutaneous)

Adjusted associations of these assessments with measures of insulin sensitivity indices obtained in Aim 1 will be explored for both groups, as described in the analysis plan for Aim 1.

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SECTION II: RESEARCH INVOLVING DRUGS, BIOLOGICS, RADIOTRACERS, PLACEBOS AND DEVICES

A. RADIOTRACERS ☒N/A

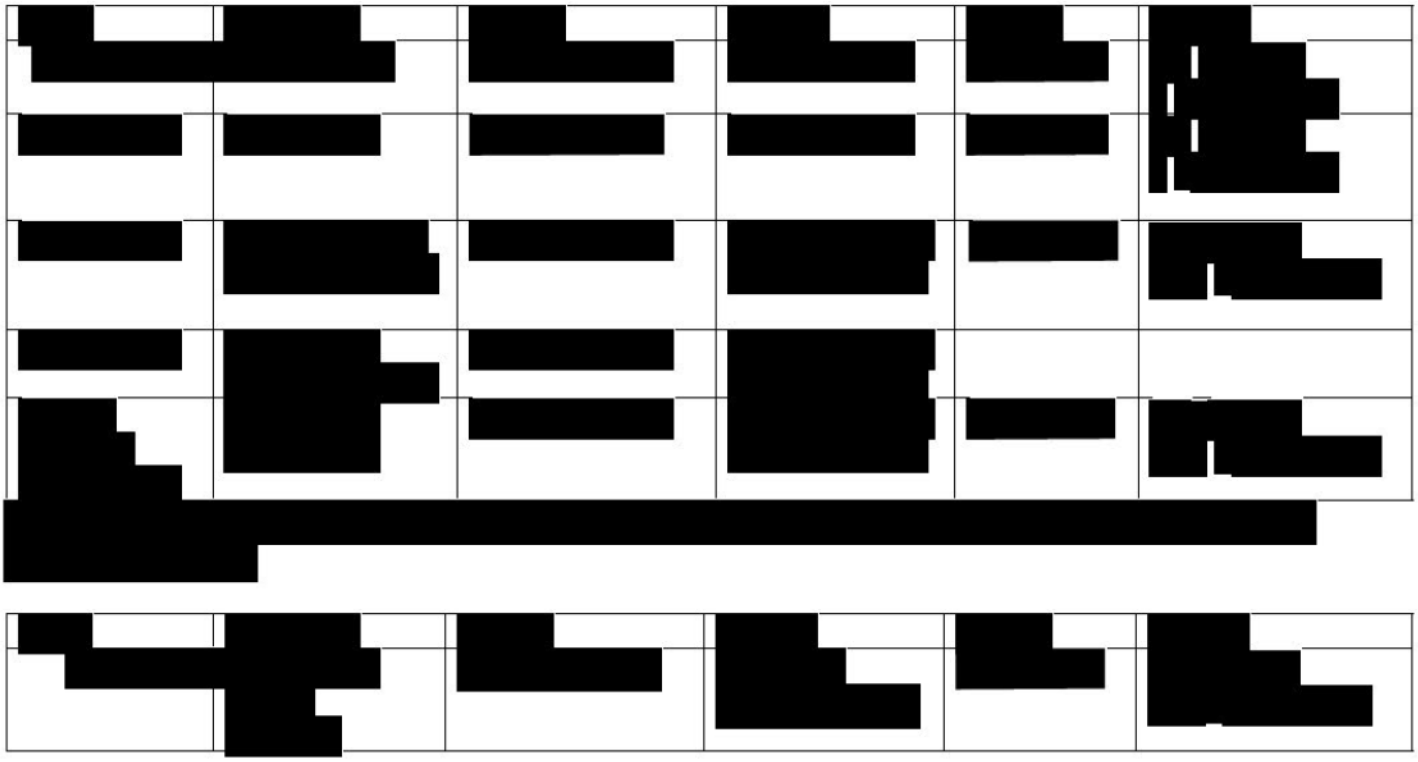
B. DRUGS/BIOLOGICS ☐N/A

1. If an **exemption from IND filing requirements is** sought for a clinical investigation of a drug product that is lawfully marketed in the United States, review the following categories and complete the category that applies (*and delete the inapplicable categories)*:

Not Applicable

2. **Background Information:** Provide a description of previous human use, known risks, and data addressing dosage(s), interval(s), route(s) of administration, and any other factors that might influence risks. If this is the first time this drug is being administered to humans, include relevant data on animal models.

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. In addition, we have included dosing from a publication from this summer, which clarifies the rationale for dosing in youth with type 1 diabetes in HIC# [redacted] and the study proposed herein.



3. **Source:** Identify the source of the drug or biologic to be used. There is no study drug (treatment) for this protocol. However, 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.

a) Is the drug provided free of charge to subjects? ☒YES ☐NO
If yes, by whom? Study funding

4. **Storage, Preparation and Use:** Describe the method of storage, preparation, stability information, and for parenteral products, method of sterilization and method of testing sterility and pyrogenicity.
The glycerol and glucose isotopes will be kept with Yale-New Haven Hospital Investigational Drug Service, 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.

Check applicable Investigational Drug Service utilized:

☒ YNHH IDS ☐ CMHC Pharmacy ☐ West Haven VA
☐ PET Center ☐ None
☐ Other:

***Note:** If the YNHH IDS (or comparable service at CMHC or WHVA) will not be utilized, explain in detail how the PI will oversee these aspects of drug accountability, storage, and preparation.*

5. **Use of Placebo:** ☒Not applicable to this research project

6. **Continuation of Drug Therapy After Study Closure** ☒Not applicable to this project

B. DEVICES ☒N/A

SECTION III: RECRUITMENT/CONSENT AND ASSENT PROCEDURES

1. **Targeted Enrollment: Give the number of subjects: 108**
   a. Targeted for enrollment at Yale for this protocol: 108
   b. If this is a multi-site study, give the total number of subjects targeted across all sites: N/A

2. **Indicate recruitment methods below.** Attach copies of any recruitment materials that will be used.

☐ Flyers ☐ Internet/web postings ☐ Radio
☐ Posters ☐ Mass email solicitation ☐ Telephone
☐ Letter ☐ Departmental/Center website ☐ Television
☐ Medical record review* ☐ Departmental/Center research boards ☐ Newspaper

☐ Departmental/Center newsletters ☐ Web-based clinical trial registries ☐ Clinicaltrails.gov
☐ YCCI Recruitment database ☐ Social Media (Twitter/Facebook):
☒ Other: Patients of the Yale Children's Diabetes Clinic

*** Requests for medical records should be made through JDAT as described at**
http://medicine.yale.edu/ycci/oncore/availableservices/datarequests/datarequests.aspx

3. **Recruitment Procedures:**

a. Describe how potential subjects will be identified. Subjects with diabetes will be identified at their regular visits to the Yale Adult Diabetes Office and the Yale Children's Diabetes Program clinic.

b. Describe how potential subjects are contacted. Contact will be made by either the P.I. or another research member via phone conversation or clinical visit at a Yale Diabetes clinic.

c. Who is recruiting potential subjects?

[redacted]

4. **Assessment of Current Health Provider Relationship for HIPAA Consideration:**

Does the Investigator or any member of the research team have a direct existing clinical relationship with any potential subject?

☐Yes, all subjects
☒Yes, some of the subjects
☐No

If yes, describe the nature of this relationship. Participants will be recruited from the Yale Children's Diabetes Program and the research team will have an existing clinical relationship with these subjects.

5. **Request for waiver of HIPAA authorization:** (When requesting a waiver of HIPAA Authorization for either the entire study, or for recruitment purposes only. Note: if you are collecting PHI as part of a phone or email screen, you must request a HIPAA waiver for recruitment purposes.)

Choose one:

☐ For entire study
☒ For recruitment/screening purposes only
☐ For inclusion of non-English speaking subject if short form is being used and there is no translated HIPAA research authorization form available on the University's HIPAA website at hipaa.yale.edu.

i. Describe why it would be impracticable to obtain the subject's authorization for use/disclosure of this data: To determine whether patients will be eligible for this study and thus consent procedures, the study team will review the medical records.

ii. If requesting a waiver of **signed** authorization, describe why it would be impracticable to obtain the subject's signed authorization for use/disclosure of this data: Some potential subjects may call or email after learning about the trial from Clinicaltrials.gov, or support groups. We can ask some basic screening questions about age, date of diagnosis etc. before scheduling an enrollment visit to do further screening to determine study eligibility.

The investigator assures that the protected health information for which a Waiver of Authorization has been requested will not be reused or disclosed to any person or entity other than those listed in this application, except as required by law, for authorized oversight of this research study, or as specifically approved for use in another study by an IRB.

Researchers are reminded that unauthorized disclosures of PHI to individuals outside of the Yale HIPAA-Covered entity must be accounted for in the "accounting for disclosures log", by subject name, purpose, date, recipients, and a description of information provided. Logs are to be forwarded to the Deputy HIPAA Privacy Officer.

6. **Process of Consent/Assent:** Describe the setting and conditions under which consent/assent will be obtained, including parental permission or surrogate permission and the steps taken to ensure subjects' independent decision-making.
   Potential participants who express interest in the study will be given a copy of the Consent Form/Parent Permission/Assent Form, asked to review, and then given the opportunity to discuss the study with the investigators in detail. The investigator will comply with all applicable regulatory and legal requirements, ICH GCP guidelines and the Declaration of Helsinki in obtaining and documenting the informed consent. It will of course be stressed that participation is voluntary, and non-participation will not affect continuing care at the Yale Children's Diabetes Program. The participant will have the right to refuse to participate or to withdraw at any time.

7. **Evaluation of Subject(s) Capacity to Provide Informed Consent/Assent:** Indicate how the personnel obtaining consent will assess the potential subject's ability and capacity to consent to the research being proposed.
   Subjects will have a face-to-face meeting with an Investigator and/or Study Personnel to address questions about the study rationale, procedures, risks and benefits. In order to identify and clarify any misconceptions, subjects will be encouraged to describe the research procedures and their associated risks in their own words, followed by correction of any errors by an Investigator or Study Personnel.

8. **Non-English Speaking Subjects:** Explain provisions in place to ensure comprehension for research involving non-English speaking subjects. If enrollment of these subjects is anticipated, translated copies of all consent materials must be submitted for approval prior to use.
   N/A

As a limited alternative to the above requirement, will you use the short form* for consenting process if you unexpectedly encounter a non-English speaking individual interested in study participation and the translation of the long form is not possible prior to intended enrollment? YES ☐ NO ☒

Note* If more than 2 study participants are enrolled using a short form translated into the same language, then the full consent form should be translated into that language for use the next time a subject speaking that language is to be enrolled.

Several translated short form templates are available on the HRPP website (yale.edu/hrpp) and translated HIPAA Research Authorization Forms are available on the HIPAA website (hipaa.yale.edu). If the translation of the short

form is not available on our website, then the translated short form needs to be submitted to the IRB office for approval via modification prior to enrolling the subject. ***Please review the guidance and presentation on use of the short form available on the HRPP website.***

If using a short form without a translated HIPAA Research Authorization Form, please request a HIPAA waiver in the section above.

9. **Consent Waiver: In certain circumstances, the HIC may grant a waiver of signed consent, or a full waiver of consent, depending on the study.** If you will request either a waiver of consent, or a waiver of signed consent for this study, complete the appropriate section below.

☐Not Requesting any consent waivers

☐Requesting a waiver of signed consent:

☐ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study (Note that an information sheet may be required.)

For a waiver of signed consent, address the following:

- Would the signed consent form be the only record linking the subject and the research? YES ☐ NO ☐
- Does a breach of confidentiality constitute the principal risk to subjects? YES ☐ NO ☐

OR

- Does the research pose greater than minimal risk? YES ☐ NO☐
- Does the research include any activities that would require signed consent in a non-research context? YES ☐ NO ☐

☒ Requesting a waiver of consent:

☒ Recruitment/Screening only *(if for recruitment, the questions in the box below will apply to recruitment activities only)*

☐ Entire Study

For a full waiver of consent, please address all of the following:

- Does the research pose greater than minimal risk to subjects?
  - ☐ Yes *If you answered yes, stop. A waiver cannot be granted.*
  - ☒ No
- Will the waiver adversely affect subjects' rights and welfare? YES ☐ NO☒
- Why would the research be impracticable to conduct without the waiver? Medical record review must be conducted to determine who is eligible for potential participation
- Where appropriate, how will pertinent information be returned to, or shared with subjects at a later date? *Write here*

SECTION IV: PROTECTION OF RESEARCH SUBJECTS

Confidentiality & Security of Data:

1. What protected health information (medical information along with the HIPAA identifiers) about subjects will be collected and used for the research? Protected health information that will be collected and used for the research will include the research study records, medical records, blood sugars, results of metabolic testing, services provided in connection with this study, the entire research record and any medical records held by Yale New Haven Hospital and the Yale School of Medicine, medical history of diabetes and other conditions that may affect eligibility for study participation.

How will the research data be collected, recorded and stored? Subjects will be assigned an identifier that will be used instead of their name.
Clinical research forms will be created to allow for collection of data from schools and for study visits. Our study team is very experienced in collecting data and maintaining study databases, as we regularly conduct clinical trials. Study records will be maintained in 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.

2. How will the digital data be stored? ☐CD ☐DVD ☐Flash Drive ☐Portable Hard Drive ☒Secured Server ☐Laptop Computer ☐Desktop Computer ☐Other

3. What methods and procedures will be used to safeguard the confidentiality and security of the identifiable study data and the storage media indicated above during and after the subject's participation in the study? The list of names of the study subjects and their assigned code numbers will be kept in a locked file cabinet or password-protected computer file by study team members.

   Individual patient medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. Such medical information may be given to the patient's personal physician or to other appropriate medical personnel responsible for the patient's welfare.

   All individuals involved in the conduct of this study will be trained on HIPAA privacy regulations and will receive human subject protection training. The principal investigator will periodically monitor the methods and procedures described herein to ensure proper use and that continued protections are in place and being followed.

All portable devices must contain encryption software, per University Policy 5100. If there is a technical reason a device cannot be encrypted please submit an exception request to the Information Security, Policy and Compliance Office by clicking on url http://its.yale.edu/egrc or email it.compliance@yale.edu

4. What will be done with the data when the research is completed? Are there plans to destroy the identifiable data? If yes, describe how, by whom and when identifiers will be destroyed. If no, describe how the data and/or identifiers will be secured. . 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. Data will be de-identified at end of study prior to study termination.

5. If appropriate, has a Certificate of Confidentiality been obtained? *N/A*

SECTION V: POTENTIAL BENEFITS

Potential Benefits: Identify any benefits that may be reasonably expected to result from the research, either to the subject(s) or to society at large. (Payment of subjects is not considered a benefit in this context of the risk benefit assessment.)

Aims 1 and 2 focus on characterization of effects of obesity and puberty on metabolism in youth with T1D and do not have direct short-term benefits to the participants.

Long-term benefits of this research are a generalizable understanding of the changes of insulin resistance related to obesity and puberty in youth with T1D, which will help to inform future treatment strategies.

SECTION VI: RESEARCH ALTERNATIVES AND ECONOMIC CONSIDERATIONS

1. **Alternatives:** What other alternatives are available to the study subjects outside of the research?
   The alternative to participating in this study is non-participation. Subjects who choose not to participate may continue their normal care in the Yale Pediatric Diabetes Program. There are no risks of non-participation.
2. **Payments for Participation (Economic Considerations):** Describe any payments that will be made to subjects, the amount and schedule of payments, and the conditions for receiving this compensation.
   Participants will receive $200 for an overnight hospital stay and completion of an insulin clamp and DEXA scan, $75 for completion of MRI. Compensation will be distributed via a reloadable Bank of America card per university policy.
3. **Costs for Participation (Economic Considerations):** Clearly describe the subject's costs associated with participation in the research, and the interventions or procedures of the study that will be provided at no cost to subjects.
   The study will be paying for all of the costs related to the study visits.
4. **In Case of Injury:** This section is required for any research involving more than minimal risk, and for minimal risk research that presents the potential for physical harm (e.g., research involving blood draws).

   a. Will medical treatment be available if research-related injury occurs? *Yes*

   b. Where and from whom may treatment be obtained? From the research staff or YNHH if necessary
   c. Are there any limits to the treatment being provided? Injuries caused directly by participation in the study. There will be no payment for lost wages and/or direct or indirect losses.
   a. Who will pay for this treatment? Subjects will be responsible for the costs of such medical care that is not covered by their own health insurance.
   d. How will the medical treatment be accessed by subjects? By notifying Research Team members

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IMPORTANT REMINDERS

Will this study have a billable service? **Yes ☐** **No ☒**

A billable service is defined as any service rendered to a study subject that, if he/she was not on a study, would normally generate a bill from either Yale-New Haven Hospital or Yale Medical Group to the patient or the patient's insurer. The service may or may not be performed by the research staff on your study, but may be provided by professionals within either Yale-New Haven Hospital or Yale Medical Group (examples include x-rays, MRIs, CT scans, specimens sent to central labs, or specimens sent to pathology). Notes: 1. There is no distinction made whether the service is paid for by the subject or their insurance (Standard of Care) or by the study's funding mechanism (Research Sponsored). 2. This generally includes new services or orders placed in EPIC for research subjects.

If answered, "yes", this study will need to be set up in OnCore, Yale's clinical research management system, for Epic to appropriately route research related charges. Please contact oncore.support@yale.edu

Are there any procedures involved in this protocol that will be performed at YNHH or one of its affiliated entities? **Yes ☒ No ☐**

If Yes, please answer questions a through c and note instructions below.

a. Does your YNHH privilege delineation currently include the **specific procedure** that you will perform? **Yes ☒ No ☐**

b. Will you be using any new equipment or equipment that you have not used in the past for this procedure? **Yes ☐ No ☒**

c. Will a novel approach using existing equipment be applied? **Yes ☐ No ☒**

If you answered "no" to question 4a, or "yes" to question 4b or c, please contact the YNHH Department of Physician Services (688-2615) for prior approval before commencing with your research protocol.

IMPORTANT REMINDER ABOUT RESEARCH AT YNHH

Please note that if this protocol includes Yale-New Haven Hospital patients, including patients at the HRU, the Principal Investigator and any co-investigators who are physicians or mid-level practitioners (includes PAs, APRNs, psychologists and speech pathologists) who may have direct patient contact with patients on YNHH premises must have medical staff appointment and appropriate clinical privileges at YNHH. If you are uncertain whether the study personnel meet the criteria, please telephone the Physician Services Department at 203-688-2615. **By submitting this protocol as a PI, you attest that you and any co-investigator who may have patient contact has a medical staff appointment and appropriate clinical privileges at YNHH.**

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