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PROTOCOL TITLE:
Impact of Meal Timing on Glycemic Profile in Latino Adolescents with Obesity.

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STUDY ABSTRACT

In adolescents, conventional obesity treatment comprehensively addresses nutritional, activity, and behavioral topics. Due to limited resources in historically marginalized communities, implementation of nutrition-based interventions that require easy access to fresh food and ability to change the home environment is difficult, which may exacerbate health disparities. It is critical to find nutrition strategies and recommendations that are impactful, sustainable, and cost effective across all communities. There is growing interest in time-based interventions focusing on "when" food is consumed rather than on prescribed macronutrient composition. Time-restricted eating (TRE) is a type of meal-timing which involves fasting for at least 14-hours per day and eating over a 10-hour eating window initiated in the morning, mid-day, or afternoon. TRE recommendations are simple in merely dictating when eating occurs and thus may represent a more straightforward approach for adolescents than other caloric restriction regimens relying on numeracy (kilocalories and macronutrients) and goal setting. In adults, early-day TRE has been shown to reduce body weight, fasting glucose, and insulin resistance. By contrast, restricting food intake to the evening has produced mostly null results or even worsened post prandial glucose levels and β-cell responsiveness. To date, there has been no trial comparing early vs. late TRE on glycemic profiles in adolescents, and it is unclear how meal-timing impacts glycemic profiles in youth. The optimal timing of food intake for adolescents may be very different than adults due to increasing sex steroids and growth hormone levels overnight which may contribute to increased insulin resistance in the early morning. The proposed proof-of-concept study addresses this question by measuring metabolic response to a test meal consumed in the morning, afternoon, and evening among 30 adolescents with obesity using a within participant design. These findings will provide the needed research base for the refinement of TRE interventions in adolescence.

SPECIFIC AIMS

The prevalence of type 2 diabetes in adolescents is steadily increasing, specifically among Latino youth. Unfortunately, there is a lack of effective preventative treatments available

to delay the progression to type 2 diabetes in youth living with obesity and pre-diabetes. Specifically, it is challenging for many adolescents to maintain adherence to multicomponent interventions resulting in high attrition which then decreases efficacy. There is growing interest in time-based interventions focusing on "when" food is consumed rather than on prescribed macronutrient composition. In the adult population there has been increasing evidence that **time-restricted eating (TRE)**, which consists of limiting daily food intake to an 8 to 10-hour period or less may improve insulin sensitivity and β-cell responsiveness. However the results of TRE in adults appear to depend on when eating occurs. Early TRE (last eating event prior to 16:00) has been shown to reduce body weight, fasting glucose and insulin, and insulin resistance. However, restricting food intake to the evening produced mostly null results or worsened post prandial glucose level. One possible explanation is that consuming food early in the day synchronizes the central and peripheral circadian clocks involved in energy expenditure and fat oxidation and minimizes glycemic excursions and endogenous glucose production via enhanced insulin secretion. It has been proposed that the enhanced insulin secretion may be secondary to corresponding diurnal variations in the release of incretin hormones such as glucagon, glucagon-like-peptide-1 (GLP-1), glucose-dependent insulinotropic polypeptide (GIP), and pancreatic peptide (PP).

Because of its simplicity, TRE may represent a more feasible approach for adolescents than other caloric restriction regimens. Our team recently conducted the first study examining the feasibility of TRE in adolescents with obesity. In this study, fifty adolescents were randomized to a self-selected 8-hour TRE compared to a prolonged eating window. TRE was found to be feasible, safe, and acceptable but there was no difference in glycemic profiles or weight loss between groups. These null findings may be due to the late eating window that was selected. To date, there have been no trial comparing early vs. late TRE on glycemic profiles in adolescents and it is unclear how meal-timing impacts glycemic profiles in youth. It remains unknown how the timing of meal consumption directly effects glycemic profiles and cardiometabolic endpoints in this age group. Thus, to address this question, in our proof-of-concept study, we aim to measure glycemic and metabolic responses to a test meal (controlled for macronutrient profile and caloric amount) at various meal-timings (early vs. afternoon vs. late) in thirty Latino adolescents (ages 13-19 years), with obesity, without diabetes. All participants will consume three standard test meals administered in random order at different times of day over a two-week period: (1) Early: test meal consumed at 8 AM; (2) Afternoon: 12 PM; (3) Late: 4 PM. Test meals will be consumed on different days at least three days apart after a minimum of a 16-hour fasting period. A continuous glucose monitor (CGM) will be placed on the participant for the duration of the 2-week period. The **primary endpoints** will be frequently sampled glucose, insulin, and c-peptide area under the curve (AUC) collected after consumption of a standard test meal, insulinogenic index (IGI: change in insulin/change in glucose over the first 30 min after the load), and glucose variability as captured by percent time in range on CGM. We **hypothesize** that adolescents with obesity will have reduced AUC and IGI following the later meals compared to the early meal. We will test these hypotheses with the following aims:

Aim 1: Identify how timing of meal consumption after a prolonged 16-h fast affects glucose and insulin response in Latino adolescents with obesity. We will measure insulin secretion, clearance rates, and sensitivity assessed with a) 3-h frequent sampling of glucose, insulin, and c-peptide post meal, b) calculated insulinogenic index, and c) percent time in range measured by CGM. ***We expect*** that meal consumption at 12 and 4 PM will be associated with lower post prandial glucose excursions, decreased AUC and IGI, and increased percent time in range compared to that same meal consumed at 8 AM.

Aim 2: Examine the association between timing of meal consumption and incretin and pancreatic hormones in Latino adolescents with obesity. We will collect frequent sampling of glucagon, total GLP-1, GIP, and PP concentrations post-meal to explore how diurnal variation in these hormones impact insulin sensitivity. **We expect** that meal consumption at 12 and 4 PM will in greater change in incretin and pancreatic hormones concentrations compared to that same meals consumed at 8 AM.

Future Direction: The data collected during this pilot will inform the design of a randomized clinical trial to test the feasibility, safety, and efficacy of early vs. late TRE in adolescents with obesity to reduce glycemic and insulin response for the prevention of type 2 diabetes.

RESEARCH STRATEGY

i. Significance:

In the United States, 1 in 5 adolescents has obesity, and 30-50% of those go on to develop early onset type 2 diabetes. Increased pediatric obesity has been accompanied by a rising incidence of type 2 diabetes in adolescents, specifically among Latino youth. With increasing prevalence and increasing cost for care, obesity in adolescents is expected to result in extensive financial costs, and significant life limiting complications. In adolescents, conventional obesity treatment comprehensively addresses nutritional, physical activity, and behavioral topics with the goal of achieving clinically meaningful weight loss. Adherence to comprehensive lifestyle interventions is challenging for adolescents, in part because these approaches require frequent monitoring and engaging in multiple behavioral targets continuously. Therefore, there is a growing interest in simplifying intervention recommendations to decrease reliance on numeracy (Kilo calories and macronutrients), goal setting and complex skillsets. One simpler and promising approach is based on timing of eating.

Time-restricted eating (TRE) involves shortening the eating window to a pre-specified number of hours per day and fasting for the remaining of the day without necessarily altering diet quality and quantity. TRE has been shown to promote weight loss, improve fasting glucose and postprandial glucose levels, mean daily glucose, and insulin resistance, as well as blood triglyceride, total cholesterol, LDL cholesterol levels and overall well-being**.** Because of its simplicity, TRE may represent a more feasible approach for adolescents because it removes the need for intensive counting of daily caloric intake and focuses on a straightforward task of consuming food during a pre-specified time. Our

preliminary data, collected in 50 adolescents, shows the feasibility, acceptability, and safety of implementing 8-hour TRE in adolescents.

The results of TRE in adults appear to depend on when eating occur. Early TRE (last eating event prior to 16:00) has been shown to reduce body weight, fasting glucose and insulin, insulin resistance, and inflammation. However, restricting food intake to the afternoon and/or evening produced mostly null results or worsened post prandial glucose levels and β-cell responsiveness. Thus, many adult trials have promoted TRE early in the day because it has been hypothesized to synchronize the central and peripheral circadian clocks involved in energy expenditure and fat oxidation and minimizes glycemic excursions and endogenous glucose production. In adults, previous evidence has shown glucose tolerance, skeletal muscle fatty acid oxidation, and diet-induced thermogenesis are higher in the morning than in the evening and that an evening meal induces higher postprandial glucose concentrations and different secretion of insulin and incretins compared with the same meal consumed in the morning. These findings suggest that eating earlier in the daytime might be more optimal for food consumption in adults, whereas delayed eating may predispose to more metabolic dysfunction. It has been proposed that the enhanced insulin secretion seen in response to a morning meal may be secondary to corresponding diurnal variations in the release of incretin hormones such as glucagon-like peptide 1 and glucose-dependent insulinotropic peptide.

An early eating window may not align with many adolescents' development and social schedules that often shift their food consumption to later in the day. This shifting chronotype, coupled with increasing sex steroids and growth hormone levels overnight, has been shown to cause increased insulin resistance in the early morning for this age group, contrary to what is seen in adults. Given the changing metabolic state of many adolescents living with obesity, it remains unknown how the timing of meal consumption directly effects glycemic profiles and cardiometabolic endpoints in this age group. In the proposed proof-of-concept study we aim to measure glycemic and metabolic responses to a test meal (controlled for macronutrient profile and caloric amount) consumed in the morning, afternoon or evening using a cross-over design in 30 Latino adolescents with obesity. These findings will provide metabolic evidence to inform what eating window is most appropriate for adolescents with obesity utilizing TRE.

ii. Innovation:

The proposed study is theoretically and clinically innovative for the following reasons: **This is the first study to evaluate the relationships between the timing of food intake and insulin sensitivity in adolescents with obesity.** This study will determine if the timing of the first meal of the day alters the glycemic profile in adolescents with obesity. These findings will provide metabolic evidence to guide the recommendation of when time-based interventions should occur in this age group as a non-pharmacological intervention to delay conversion to type 2 diabetes. **New insight into the relationship between insulin secretion and incretin release in adolescents.** By evaluating the difference in incretin hormone response by time of meal consumption, this study will investigate the relationship between insulin secretion, incretin

release, and diurnal variation in adolescents with obesity. **Expand our understanding of the use of CGM in adolescents with obesity**. There is very limited experience of the use of CGM among underserved racial/ethnic minority populations at risk for type 2 diabetes. Current CGM-based metrics provide a clinically meaningful macroscopic perspective on an individual's glycemia. This study aimed to gain data-driven insights from the postprandial glucose response to meal consumption at various times throughout the day to determine if changes in glucose peaks shift by the time of first meal consumption. Results from this study could potentially change the way adolescents with obesity are treated in the future by incorporating meal-timing behaviors as part of their medical regimen.

PROCEDURES INVOLVED

Overview of Proposed Study Design: We propose a cross-over, proof-of-concept study to measure glycemic and metabolic responses to a test meal (controlled for macronutrient profile and caloric amount) administered at various times of the day (early vs. afternoon vs. late) in thirty Latino adolescents (ages 13-19 years), with obesity, without diabetes, to determine how timing of eating impacts glycemic response to the test meal after a 16-h fasting period. All participants will consume three standard test meals administered in random order at different times of day over two-weeks: (1) Early: test meal consumed at 8 AM; (2) Afternoon: test meal consumed at 12 PM; (3) Late: test meal consumed at 4 PM. A continuous glucose monitor (CGM) will be placed on the participant for the duration of the 2-week period. Baseline and post-meal samples will be assayed for glucose, insulin, c-peptide, GLP-1, GIP, PP at times -10, , 10, , 30, , 60, 90, 120, and 180 minutes after glucose. The proposed protocol is modified to include multiple samples during the initial 30 minutes when glucose is rapidly increasing, allowing for modeling of glucose stimulated insulin secretion. The primary endpoints will be glucose, insulin, and C-peptide area under the curve, insulinogenic index, and glucose variability as captured by percent time in range on CGM following the test meal.

The implementation steps of the proposed RCT are as follows: **(1)** The staff will introduce the study to all eligible participants either in person or virtually and consent interested families for the study; **(2)** All participants and their families will complete baseline study surveys in REDcap; **(3)** All participants and their families will receive training on the use and application of the CGM, which is FDA approved in patients 2 years and older. All equipment required for the duration of the study will be distributed to the participants in-person. **(4)** All participants will be randomized to the order in which they consume their test meal. Test meals will be consumed on different mornings at least three days apart after a 16-h fasting period. Outside of study visits, participants will purchase all their meals as they would normally without alternation to food quality or quantity; **(5)** The study staff will conduct study assessments with participants at baseline and during each test meal study visit (3 total). Participant will be given the option to redo visits with missing lab results (up to 6 total); **(6)** The study staff will perform weekly phone encounters with the participants to assess barriers to study visit attendance and to record any

medication changes or health issues that have occurred in the last 7 days; **(7)** The PI and research team will meet bi-weekly to monitor all study procedures and oversee data management and analysis.

The implementation steps of the proposed test meals and blood collection are as follows:

1) Subjects without a port will have an IV inserted;
2) -10 min pre-meal blood sample (5 mL) will be collected from the port or IV for fasting labs;
3) Subjects will consume meal; and
4) 10 min post-meal blood sample (5mL), 30 min post-meal blood sample (5mL), 60 min post-meal blood sample (5mL), 90 min post-meal blood sample (5mL), 120 min post-meal blood sample (5mL), and 180 min post-meal blood sample (5mL) will be collected from the port or IV post-meal test.

Sharing of Testing Results with Subjects

Study endpoints will be shared with participants upon request via email or over the phone. The PI will review any tests results and if additional medical evaluation nis warranted will refer to the primary care providers.

Study Timelines

Intervention: For the 14 days study period, all participants will maintain their usual lifestyle, including food consumption, physical activity, and sleep patterns. Physical activity and sleep recommendations consistent with the American Academy of Pediatrics guidelines for adolescents will be encouraged but not formally prescribed. **Test Meal Composition:** Each participant will consume three test meals over the study period, administered at three different times of the day. The test meals will be prepared by the dietetic staff at CHLA. The test meal will consist of 770 calories total (one-third of the recommended total daily caloric intake for age, 30% protein, 10% fat, and 60% carbohydrates [CHO]). The test meal will be comprised of a deli sandwich (560 calories, 84 grams of CHO, 10 grams of added sugar) snack bar (100 calories, 17 grams of CHO, 9 grams sugar), and Greek yogurt (110 calories, 16 grams CHO, 7 grams sugar). **Timing of Test Meal Consumption:** There will be three time periods in which the test meal is administered over the study period: 8 AM, 12 PM, and 4 PM. To mimic a TRE model, in which the daily fasting period is extended to 14-16 hours or more all participants will be asked to fast for at least 16-hs prior to their test meal visit. Participants will be asked to not consume any food for 3 hours after the test meal is completed. **Continuous Glucose Monitor**. All participants will be trained to wear a blinded continuous glucose monitor sensor using manufacturer educational materials under the supervision of research staff. Participants will be asked to wear the CGM for 14 days after enrollment. During each study visit, the CGM reader will be connected to the site database to create an individual participant report.

Study Timeline	Q1	Q2	Q3	Q4
Study Start Up				
Hire and train study personnel	X			
Finalize protocol, obtain Institutional Review Board (IRB) approval	X			
Data & document review	X			
Design Test Meal	X			
Apply for CHLA CTSI Biostats Core Pilot Grant	X			
Create Testing Protocol with CTU	X			
Study implementation	X	X	X	
Data Handling				
Data entry/cleaning and analysis		X	X	X
Publications and presentations			X	X
Future Grant Preparation				
R01 and ADA				X

Measurements: The study team will conduct all assessments baseline and each meal consumption visit (3 total). Participant will be given the option to redo visits with missing lab results (up to 6 total). All data will be collected and stored in REDCap.

Aim 1: Identify how timing of meal consumption after a prolonged 16-h fast affects glucose and insulin response in Latino adolescents with obesity.

Quantifying glycemic profile after test meal from venous sample: Baseline and post-meal samples will be assayed for glucose, insulin, and c-peptide at times -10, , 10, , 30, , 60, 90, 120, and 180 minutes after the meal is consumed. The proposed protocol is modified to include multiple samples during the initial 30 minutes when glucose is rapidly increasing, allowing for modeling of glucose stimulated insulin secretion. Using this approach to model glucose kinetics without the need to administer insulin is a safe and cost-effective surrogate of β-cell function and can be more easily implemented in youth. Insulinogenic index (change in insulin/change in glucose over the first 30 min after the load) will be calculated. IGI has been widely used as an index of early phase insulin secretion in clinical studies. It is highly correlated with the acute insulin response on intravenous glucose tolerance test and is considered a reasonable surrogate.

Quantifying glycemic profile on CGM: Four parameters will be computed from CGM data for each test meal segment: Starting glucose: The glucose value at the start of the meal. Time to peak: The time difference between the start of the meal response and the meal response peak in minutes. Maximum glucose rise: The difference between the glucose levels at the start of the meal and post-meal peak in mg/dL. Glucose incremental area under curve: The positive

area under the post-meal glucose curve after subtracting the glucose value at the start. We will capture glucose readings every 5 minutes and obtain the weekly %TIR over the course of the study.

Aim 2: Examine the association between timing of meal consumption and incretin and pancreatic hormones in Latino adolescents with obesity to explore if the enhanced insulin secretion is secondary to corresponding variations in the release of incretin hormones.[51]

Quantifying incretin hormones after test meal from venous sample: Following baseline sampling (0 min), a test meal will be administered. glucagon, GLP-1, GIP, and PP concentrations will be measured in duplicate from plasma samples using Millipore Enzyme-linked Immunosorbent Assays (ELISA) or multiplex assays according to the recommendations of the manufacturer (Birmington, MA). Total area under the cure (AUC) will be calculated for venous glucagon, GLP-1, GIP, and PP concentrations using the trapezoid methods using GraphPad Prism (Version .01, GraphPad Software Inc, San Diego, CA, USA).

Statistical Consideration: The sample size estimates, and analytical plans were developed under the guidance of Dr. Ramon Durazo-Arvizu who is the Director of the TSRI Biostatistics core and who will also be a mentor on this award. Descriptive statistics, including graphical depictions, will be utilized to assess the degree of symmetry/normality of continuous outcomes and identification of outliers. A ladder of powers approach will be applied to identify the best easy-to-interpret transformation of the continuous outcome variable. The statistical approach will be implemented with and without transformations when appropriate. Data for this 3-treatment, 3-period cross-over design will be analyzed using linear mixed effects model as described in the literature and implemented in STATA 17.1 (College Station, TX) Sensitivity analysis applying weighted least squares regression will follow. Areas under curves (AUC) will be calculated by the trapezoid rule for levels after meal ingestion. Separate AUC will be calculated for the early (initial 30-min) and the total (entire 300-min) responses. This separation of responses in early and total was decided based on previous findings that insulin secretion is progressively increased during these first 30 min after oral nutrient ingestion. Wilcoxon matched paired test will be used for tests of significance between variables obtained during morning, afternoon, and evening meal ingestion. Spearman regression coefficients will be obtained to estimate correlation.

Potential Challenges and Alternative Approaches: *1) **Study Design**:* We considered utilizing a comparison study based on time of day of meal consumption. However, given this is a proof-of-concept proposal we felt a cross-over design would be more feasible and inform that design of a larger trial for a future grant application. 2) ***Assessment**:* The hyperglycemic clamp and FSIGT have been the most widely reported and acceptable methods to evaluate β-cell function. However, given this is a limited resources proposal in adolescents, we elected to utilize a multiple sample approach following a test meal to characterize glycemic variability in a more

real-life scenario, which is both more cost- effective and tolerable for pediatric participants. 3) ***Intervention***: Ideally this study would be conducted as a controlled feeding study, in which all food was provided to participants over the study period. This design would ensure equal daily caloric intake and equal fasting window for all test meals. However due to resource constraints and anticipated participant burden that is outside the scope of this proposal and thus we selected to conduct this in a more real-life setting. We will adjust our analysis based on fasting window to control for length of fasting before meal consumption. 4) ***CGM Use***: We considered utilizing real-time CGM feedback during the two-week period. However, given CGM use is not standard of care we opted to utilize blinded CGM for outcome monitoring to lower participant burden and enable more clear assessment of the primary intervention component of meal-timing

Inclusion and Exclusion Criteria

- We will recruit 30 Latino adolescents (age 13-19 years at enrollment, all gender expressions) from clinical programs at CHLA. Inclusion criteria are: (1) age 13-19 years; (2) Hispanic or Latino defined by the NIH as a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race; (3)Tanner stage III and above; (4) body mass index > 95th percentile; (5) participant must be willing and able to adhere to the assessments, visit schedules, and eating/fasting periods. To limit confounding factors, individuals will be considered ineligible to participate if they meet any of the following exclusion criteria: (1) diagnosis of Prader-Willi Syndrome, brain tumor or hypothalamic obesity; (2) serious intellectual disability; (3) parent/guardian-reported physical, mental of other inability to participate in the assessments; (4) previous bariatric surgery; (5) current use of an anti-obesity or other diabetes medication (e.g., phentermine, topiramate, orlistat, GLP-1 agonist, naltrexone, buproprion, or insulin); or (6) current participation in other interventional weight loss studies. Randomization tables will be first generated using SAS and fed into the study REDCap database. Participants will be randomly allocated to the sequence of meal-times consumed over the study period.
- **Inclusion Criteria:** Participants must meet all of the following criteria to be enrolled in the trial:
    - Age 13-19 years
    - Hispanic or Latino defined by the NIH as a person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race
    - Tanner stage III and above
    - Body mass index greater than or equal to the 95th percentile
    - Willing and able to adhere to the assessments, visit schedules, eating/fasting windows
- **Inclusion of Women**: We will enroll adolescents of all gender expressions, with obesity. We expect approximately 60% of the study participants will be female.
- **Inclusion of Minorities**: The study site will make it possible to recruit an ethnically and racially diverse sample. Sixty-five percent of patients seen at CHLA self-identify as Latinx,

compared to 47.5% of the population in Los Angeles. The anticipated racial and ethnic composition of the study reflects the population at CHLA.

- **Inclusion Across the Lifespan**: Children constitute the target population for this study as we aim to evaluate the impact of meal timing on glycemic profile in adolescents. In our experience, children younger than 13 years of age and older than 21 years would require different intervention/counseling strategies. Therefore, we can develop a more consistent "age-neutral" approach if we limit the age range to 13-19 years. In addition, based on our ongoing experience, this age range incorporates the general window of late pubertal development and we will limit recruitment to participants who are Tanner stage 3 or greater to exclude the potential confounding effects of early pubertal transition. Puberty will be determined by Tanner staging during examination by a physician and included as a co-variate. The IRB has strict regulations for the approach to and documenting of assent by minors and consent by their parents/guardians. Documentation of assent/consent is done via IRB-approved study-specific forms.
- **Exclusion Criteria:** Participants will not be eligible to participate if they meet any of the following criteria:
    - Previous diagnosis of Prader Willi Syndrome, brain tumor or hypothalamic obesity
    - Serious developmental or intellectual disability
    - Diagnosis of eating disorder (e.g., anorexia nervosa, bulimia nervosa, binge-eating disorder)
    - Parent/guardian-reported physical, mental of other inability to participate in the assessments (e.g., inability to wear CGM, inability to be in the imaging modality without sedation)
    - Previous or planned bariatric surgery
    - Current planned use of an anti-obesity or T2D medication (e.g. phentermine, topiramate, orlistat, glucagon like peptide-1 agonist, naltrexone, bupropion, SGLT-2 Inhibitor, etc.) or insulin
    - Current participation in other interventional weight loss studies.

Number of Subjects

- We will recruit 30 Latino adolescents (age 13-19 years at enrollment, all gender expressions) from clinical programs at CHLA.

Recruitment Methods

- Recruitment: We will combine three recruitment pools (community outreach, general pediatric, and sub-specialty populations) to reach our goal of 120 participants over 2 years (1-2 participant per week), which is like what we have achieved in prior studies with similar funding. CHLA and its academic partner USC, has a long history of successful investigator-initiated studies in youth with obesity. The CHLA endocrinology clinic currently cares for over 500 children and adolescents with

obesity annually. We receive an additional 700 referrals annually from providers all across Southern California. Of those, 50% meet eligibility criteria for this study. The demographics of our patient population are mean age 16.9 years, 60% female, mean age of diagnosis 13.2 ± 2.3 years. Sixty-five percent of our patients self-identify as Latino, compared to 47.5% of the population in Los Angeles. We will elicit support from community partners, patient and family advocates, and patients living with obesity to ensure the study design and execution aligns with- and promotes ideas and interests of all stakeholder partners.

- The CHLA endocrinology clinic currently cares for over 250 adolescents with obesity each year. Of those, 50% meet eligibility criteria. We will also recruit from affiliate hospitals and pediatric clinics in the area. The demographics of our patient population are mean age 16.9 years, 60% female. Sixty-five percent of our patients self-identify as Latino, compared to 47.5% of the population in Los Angeles.62 At CHLA, the recruitment rate in this population is between 50-60% with a retention rate of 60-80%. Based on our recruitment history in adolescents with obesity, we anticipate a consent rate of 50-60%, retention rate of 75-80%, and recruitment of 30 adolescents within the first 9 months of the award period.
- Participants will be recruited from Children's Hospital Los Angeles (Los Angeles, CA). The typical approach will be through direct involvement with the treating physician. In their clinics, they will introduce the study to their patients and connect interested participants with the study staff. Research staff will carefully review the full consent form with the participant and parent or guardian in person or via phone, answering the participant's questions about the study. Participants will be allowed to electronically sign consent forms online using DocuSign, REDCap, or printed forms in-person. Alternatively, research staff will review medical records of potential participants in order to identify participants who may be eligible. CHLA has natural language processing algorithms that allows for rapid searches of electronic medical records to identify patients with specified criteria for recruitment into studies. Research staff will first discuss potential participants with the PI and treating physician before making contact. After the initial recruitment process, a follow-up phone call will be made by a trained recruiter within one week to answer questions and solicit participation.

Withdrawal of Subjects

Principal investigator will not withdraw participants from the study without their consent. However, the researchers may end subject's participation in this study for a number or reasons, such as if subjects' safety and welfare are at risk, if study instructions are not being followed. Adolescents who experience psychiatric emergencies will be withdrawn from the study. Adolescents who experience psychiatric emergencies such as suicidal behavior will be withdrawn from the study. If patient requests to be withdrawn from the study, they can either stop all study tasks and visit or they can select what components they no longer want to continue and that data will be drafted as missing data.

Risks to Human Subjects:

a. **Human Subject Involvement, Characteristics and Design**: In our proof-of-concept study, we aim to measure glycemic and metabolic responses to a test meal (controlled for macronutrient profile and caloric amount) at various meal-timings (early vs. afternoon vs. late) in thirty Latino adolescents (ages 13-19 years), with obesity, without diabetes, to determine how timing of eating impacts glycemic response to a test meal after a 16-hour fasting period. We will enroll 30 adolescents, of all gender expressions, aged 13-19 years, with a BMI $\geq$ 95th percentile. In this cross-over design, each participant will act as their own control. The order of the meal timings consumed will be randomized across participants. A random block stratified randomization scheme will be used. Randomization tables will be first generated using SAS and fed into the study REDCap database.

b. Study Procedures, Materials, and Potential Risks:

Sources of Material. Data sources include questionnaires, blood samples, and anthropometric data. Privately identifiable information will therefore be collected. All data will be kept confidential and used only for research purposes.

- **Questionnaires, and Medical Chart Abstractions.** Participants will be asked to complete standard/validated questionnaires data using web-based (Research Electronic Data Capture, REDCap) or paper-based forms. Demographic variables include age, race, ethnicity, marital status, education, and income level. The baseline questionnaire will also ascertain data on a variety of potential risk factors, including family history, medical history, supplement use, and medication use. We will measure weight and height. Assessments will be conducted at all three study visits.
- **Blood Collection**: We will collect, process, and store blood according to standard protocols for the hemoglobin A1c, C-peptide, insulin, and glucose levels. Blood will be collected at all three study visits and stored at -70°C. DPP-4 and Aprotinin inhibitors will be added into test tubes for analysis.

Potential Risks/Adverse Events Monitoring and Procedures Used to Minimize the Risks:

Participants will be queried about adverse events at each phone call and study visit and will additionally be instructed to report adverse events as they occur. Participants will be provided with (A) a phone number and email address to report non-urgent adverse events to the research coordinator; and (B) a phone number to directly reach the study physician, in case of serious adverse events. The study physicians will immediately refer participants to the appropriate medical care (e.g., urgent care or non-urgent care) should any pressing concerns arise during the study period. In case of emergency, participants will be instructed to call 911 before calling the study physician. Should a participant require urgent or emergency care, the study physician will follow up with the participant's healthcare provider to determine whether it is safe for the participant to continue in the trial.

Adverse events will be subsequently documented and reported as described above. The severity of the event is graded as follows:

- Mild (Grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (Grade 2): the event causes discomfort that affects normal daily activities.
- Severe (Grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (Grade 4): the patient is at risk of death at the time of the event.
- Fatal (Grade 5): the event causes death.

Risks Related to prolonged fasting prior to study meal consumption. Adverse events are unlikely as a result of participation in this study. The PI, co-investigators, and study team will monitor participants for mild symptoms associated with prolonged fasting prior to study meal consumption, such as thirst, headaches, and gastrointestinal issues. To reduce the risks of increased thirst, headaches, and gastrointestinal issues, participants will be encouraged to stay hydrated by drinking water.

Attitudes and practices around food and eating. None is anticipated. We will continuously monitor for unforeseen or unanticipated events and risks. If a participant reports unhealthy compensatory eating behavior (i.e., binge eating, excessive restraint, purging, etc.) during the research visits, we will refer them to their primary provider to have an official psychological evaluation. If we suspect that the adolescent is purging or placing themselves in immediate medical danger, we will contact their parent or guardian and create a safety plan and instruct them to go the local emergency room for evaluation. In addition, the PI will contact the primary care provider to ensure appropriate referrals for psychiatric support are obtained. Adolescents who experience psychiatric emergencies will be withdrawn from the study. Dr. Salvy is a clinical behavioral psychologist and will provide oversight, monitoring, and guidance to the PI regarding the monitoring of any adverse events related to behavior changes that may occur during the study period.

Progression to diabetes requiring escalation of care. All participants will have obesity and thus increased risk of conversion to youth onset type 2 diabetes. If during the study visits any of the biochemical analysis is suggestive of new onset type 2 diabetes, they will be referred to their primary care provider for confirmatory lab testing and referral to a pediatric endocrinologist as needed.

Risks Related to Blood Draws. A small minority of participants will likely experience transient discomfort, pain, bleeding, and/or bruising from the needle insertion and/or IV. This poses minimal risk. Infection is also possible in rare cases. This risk will be minimized by having trained nursing and phlebotomy staff use sterile techniques to draw blood.

Risks Related to Biohazards. All staff will follow the Code of Federal Regulations on the handling of biospecimens (29 CFR Part 1910-1030) and hazardous chemicals (29 CFR Part 1910-1450). Human biospecimens (serum blood collected to measure: HbA1c, C-Peptide, Insulin, and Glucose) will be collected by doctors, nurses, or other trained personnel, and the collected samples will be processed and analyzed by trained technicians. All blood samples and reagents will be handled in approved areas using established guidelines set by Children's Hospital Los Angeles Occupational Health and Safety Offices. The collected blood specimens will be documented and stored in monitored and locked facilities. Blood, chemicals, and any materials that encounter biospecimens will be disposed of in designated biohazard waste receptacles, following federal and local biohazard regulations. All staff members who will encounter blood samples and chemical reagents are trained to safely handle these biospecimens and are retrained annually.

Risks Related to Privacy and Confidentiality. To protect privacy, each study participant will be assigned a unique identification (ID) number. Data forms, participant information, and biological specimens will be coded using these ID numbers. Personal, identifiable information will not appear on these materials; instead, the key linking the participant's identity to their unique identification number will be stored separately in a secure location. Records that identify study participants will be kept confidential as required by law, and every effort will be made to maintain the confidentiality of participants' study records. Except when required by law or if necessary, to protect their rights or welfare, study participants will not be identified by name or any other identifying characteristics in records disclosed outside of the investigational team. To manage study data, we will use REDCap, a secure web application that meets both HIPAA and 21 CFR Part 11 requirements. REDCap leaves a pristine audit trail by documenting all changes to data, and data access is strictly controlled with password authentication and user controls. Data from the medical chart and clinical outcomes and adverse events will be electronically uploaded to the database, and all questionnaires will be coded into and administered through REDCap. Such electronically stored data will be protected through stringent security measures assured by Children's Hospital Los Angeles technical departments and with coded ID numbers and electronic security systems required by HIPAA. Paper documents will be scanned and saved on each site's secure network and stored in locked cabinets in locked offices. Biological specimens will be stored and analyzed in locked areas with restricted access. Access to participants' data and biological specimens will be limited only to the study's investigators, clinical support staff for this study, the overseers of clinical facilities, and the study sponsor—all on a need-to-know basis. The study team has significant experience with the operation of clinical trials and safeguarding confidentiality

Trauma, Bullying, and Domestic Violence Risks. None anticipated. We will continuously monitor unforeseen or unanticipated events and risks. If trauma and/or domestic violence is suspected, either through completion of the questionnaires or through participants' report or staff

observation, we will implement the following procedures: (1) we will make participants aware of resources available to them in their communities, such as shelters; (2) we will work with participants to develop a safety plan for addressing violence and seeking shelter; and (3) we will ascertain risk to the child(ren) in the household. If we suspect that the child(ren) has/have been abused or witnessed domestic violence, we will report the incident to child protective services. Participants who are victims of domestic violence will be allowed to remain in the study if they wish to do so.

Suicidal Behavior Risks. None anticipated. We will continuously monitor for unforeseen or unanticipated events and risks. We follow the Substance Abuse and Mental Health Services Administration SAFE-T: Suicide Assessment Five-Step Evaluation and Triage approach to determine risk and the appropriate clinical response. The five steps are Identify Risk Factors, Identify Protective Factors, Conduct Suicide Inquiry, Determine Risk Level/Intervention, and Document (which includes intervention and follow-up). As per practice guidelines, we will estimate suicide risk as low, moderate, or high. All potentially suicidal participants will be given an emergency plan with numbers to call (e.g., local hospital emergency rooms, suicide hotlines, staff, and study physicians). Each plan will be individually tailored to the needs and resources of the participants. Family members will be incorporated into the plan. Adolescents who experience psychiatric emergencies such as suicidal behavior will be withdrawn from the study.

Alternative Treatments. Participants will be informed that the alternative option is not to enroll in the trial and their decision whether to enroll will not affect the quality or extent of medical care that they receive.

Handling Incidental Findings. If any clinically abnormal result or illness is uncovered, or if participants exhibit signs of depression or mental illness, the affected participant will be notified and referred to his/her physician or an appropriate health professional for treatment. Participants will be offered copies of any tests or incidental findings.

2. Adequacy of Protection Against Risk

a. Informed Consent: Participants will be recruited from Children's Hospital Los Angeles (Los Angeles, CA). The typical approach will be through direct involvement with the treating physician. In their clinics, they will introduce the study to their patients and connect interested participants with the study staff. Research staff will carefully review the full consent form with the participant and parent or guardian in person or via phone, answering the participant's questions about the study. Participants will be allowed to electronically sign consent forms online using DocuSign, REDCap, or printed forms in-person. Alternativley, research staff will review medical records of potential participants in order to identify participants who may be eligible. CHLA has natural language processing algorithms that allows for rapid searches of

electronic medical records to identify patients with specified criteria for recruitment into studies. Research staff will first discuss potential participants with the PI and treating physician before making contact. After the initial recruitment process, a follow-up phone call will be made by a trained recruiter within one week to answer questions and solicit participation. Consent can be withdrawn from at any time. Potential participants will have the following carefully explained: (1) the investigational nature of the research; (2) the objectives of the research; (3) the procedures involved in the research; (4) any and all risks and discomforts due to participation; and (5) alternative options to participation that are currently available. Clarification questions will then be elicited from the potential participants and their parents/guardians. Only after the above will an IRB-approved document recording informed consent be signed as per institutional guidelines with a copy to be given to the participant and the original stored with the research record in a secure location. Data protection will be ensured by storing on an IS-approved university database on encrypted servers. All participants will be issued a study ID with data entered in de-identified manner. All databases will be password-protected with access only to the study team. All paper/documentation/research charts will be maintained in locked locations accessible only to the study team.

b. Protection Against Risk:

Safety Reviews: The PI (Vidmar) and Primary Mentors (Goran, Raymond), and Advisory Committee (Salvy, Espinoza and Durazo-Arvizu) will review this protocol on a continuing basis for participant safety. The annual reports will include a list of adverse events and will address: (1) whether adverse event rates are consistent with pre-study assumptions; (2) reasons for dropouts from the study; (3) whether all participants met entry criteria; and (4) whether continuation of the study is justified on the basis that additional data are needed to accomplish the stated aims of the study.

Monitoring plan: Data monitoring will focus on *performance* (participant follow-up and retention, participant privacy and confidentiality, protocol adherence, and data quality) and *safety* (adverse events and required action/response). The PI and Mentoring team will be responsible for the regular monitoring of data and safety issues and will maintain the record of any reported adverse events and other concerns. The study data safety and monitoring plan will entail several components, overseen by the PI:

1. All CHLA IRB policies and continuous reporting requirements will be followed in conducting the study. Any actions taken by the IRB as part of its continuing review will be immediately reported to the NIH.
2. The PI will implement procedures to ensure that each participant provides informed consent, and that all data remain confidential. This will also include a rigorous data management protocol to optimize data entry, accuracy/checking, and retrieval. Data will be stored in password-protected files accessible only to the investigators and

staff under their supervision. In the database, only an ID code number will identify subjects. A list linking names and other identifiers with their ID codes will be stored in a separate file with a separate password. All original paper surveys and case report forms will be stored in locked file cabinets. The PI will review all data collection forms for completeness and accuracy and for protocol compliance. In the case of a breach of confidentiality or other adverse event, the PI will report the event to the IRB and the appropriate NIH officials, and appropriate procedural changes will be implemented to prevent future breaches or adverse events.

3. An internal committee comprised of the PI, Mentoring team, and consultants will meet quarterly to assess participant recruitment, accrual, and retention; data quality and timeliness; participant risk versus benefit; and the development of external conditions that could potentially affect the study.

All personnel who will interact with subjects will receive training on adverse event reporting. In response to any adverse event, forms will be completed promptly and returned to the study physicians for review and implementation of an appropriate action plan.

Potential Benefits of the Proposed Research to Research Participants and Others

Research participants may expect benefits from learning about their health. All participants will be offered copies of test results from procedures performed during the study. In the case of an abnormal test result or other incidental finding, participants will be instructed to visit their physician or other appropriate health care provider. Finally, participants may benefit from better understanding nutritional strategies. Overall, this clinical trial may inform future interventions for adolescents with obesity.

As the known risks to participants from the intervention (i.e., increased thirst, headaches) and procedures like blood draws (e.g., bruising, temporary pain) are minor, we believe that these benefits outweigh the risks, as the risks to participants are minimal and will be monitored by regularly assessing participant safety

4. Planned Inclusion Enrollment Report

Racial Categories	**Ethnic Categories**				
	Not Hispanic or Latino		**Hispanic or Latino**		**Total**
	Female	**Male**	**Female**	**Male**	
American Indian/Alaska Native	**0**	**0**	**0**	**0**	
Asian	**0**	**0**	**0**	**0**	

Native Hawaiian or Other Pacific Islander	**0**	**0**	**0**	**0**	
Black or African American	**0**	**0**	**2**	**2**	**4**
White	**0**	**0**	**10**	**6**	**16**
More than One Race	**0**	**0**	**6**	**4**	**10**
Total	**0**	**0**	**18**	**12**	**30**

Dissemination Plan:

Public Access Plan

We will comply with the NIH Policy on the Dissemination of NIH-Funded Clinical Trial Information in Notice NOT-OD-16-149. Dr. Vidmar will register the trial in ClinicalTrials.gov no later than 21 days after the first subject is enrolled. The consent form will state that information on the trial will be posted on the ClinicalTrials.gov website and that no information that can identify any participants will be posted. The specific wording will be: "A description of this clinical trial will be available on ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time." We will report summary results (including adverse events) no later than one year after the completion date. Children's Hospital Los Angeles has internal policies in place to ensure that all clinical research at the university complies with the dissemination requirements in NOT-OD-16-149.

Dissemination to the Scientific Community and Health Care Professionals

Our dissemination plan is four-pronged. We aim to generate scientifically interesting data for the scientific community, as well knowledge that can be readily scaled-up and exported for professionals and other end-users. First, findings from this study will be presented at key scientific meetings. I will develop poster presentations under the supervision of my mentor committee. Targeted forums include annual scientific meetings focusing on diabetes and obesity management, including American Diabetes Association, Endocrine Society, Pediatric Endocrine Society, and The Obesity Society. Once the study is underway, we will publish interim findings and summaries and, subsequently, the final findings and reports. The most appropriate scientific outlets of the study findings will be journals dedicated to obesity, diabetes, and lifestyle and nutrition interventions more broadly (e.g., Obesity, Pediatric Obesity, International of Obesity, Diabetes Care, American Journal of Clinical Nutrition). Third, study results will be shared with researchers and professionals interested in diabetes and obesity. We expect that these findings may be of greatest interest to colleagues who provide care to adolescents with obesity. Finally, aside from dissemination to the scientific community and health professionals, we intend to generate knowledge that can readily be integrated into the refinement of widely available,

commercial weight management programs already available and used by youth with obesity that incorporate time-based recommendations. The long-term goal of this research is to generate knowledge regarding how time-based dietary programs can benefits pediatric patients with obesity. This work can inform the development of potentially low-cost, scalable, and sustainable interventions to optimize treatment recommendations for this high-risk population.

Costs to Subjects

There will be no cost to subjects. Subjects will not be accountable for any research costs.

Consent Process

The implementation steps of the proposed randomized controlled trial (RCT) are as follows:

1. Study staff will introduce the study to all eligible subjects and recruit and consent interested families.

2. All participating subjects and their parent/legal guardian/family member will receive training on the use, application, and equipment for CGM. All equipment required will be distributed to the subject, and they will download the Dexcom app onto their personal smartphone setting up an account with a codename.

3. Consented participants wear the CGM prior to being randomized to begin with one of three meal times (Early: test meal consumed at 8 AM; Afternoon: test meal consumed at 12 PM; Late: test meal consumed at 4 PM (<30% protein, 10% fat, and 60% carbohydrates [CHO]) + blinded CGM.

5. The study staff will conduct in person/virtual study assessments of subjects and their parent/legal guardian/family member at 3 time points throughout the study period.

6. The study staff will perform weekly calls to assess barriers to adherence, review CGM data and perform dietary recall.

7. The principal investigator (PI) will complete and oversee data management and analysis.

Schedule of Study Procedures	Visit 0	Visit 1	Visit 2	Visit 3
Study Visit	Consent Visit	Day 1	Day 7	Day 14
Study Window	NA	+/-7 days	+ /- 7 days	+ /- 7 days

****Consent Session**	X			
Randomization in mealtime assignment	X	X		
Weight and height measured		X	X	X
****CGM Use and Data Review**:	X	X	X	X
Time in range	X	X	X	X
Education and equipment	X			
CGM Placed	X			
****24-hour Dietary Recall Collection**		X	X	X
Research blood draw/fasting labs: *glucose, insulin, c-peptide, glucagon, GLP-1, GIP, PP: -10, minutes.*		X	X	X
Lab Testing: *at post-meal samples glucose, insulin, c-peptide, glucagon, GLP-1, GIP, PP*		X	X	X
Meal Test: 10, , 30, , 60, 90, 120, , 180 minutes		X	X	X
****Surveys**		X	X	X
Demographic information *Parent/family member/guardian*	X			
Pittsburg Sleep Index *Adolescent*		X	X	X
Satisfaction Survey *Adolescent*				X
Compensation		X	X	X

**Subjects have the option of completing these procedures virtually via WebEx or over phone. If subject and/or parent/family member/guardian opts for this option, the study team will coordinate this with the subject.

Process to Document Consent in Writing

Participants will be recruited from Children's Hospital Los Angeles (Los Angeles, CA). The typical approach will be through direct involvement with the treating physician. In their clinics, they will introduce the study to their patients and connect interested participants with the study staff. Research staff will carefully review the full consent form with the participant and parent or guardian in person or via phone, answering the participant's questions about the study. Participants will be allowed to electronically sign consent forms online using DocuSign, REDCap, or printed forms in-person. Alternatively, research staff will review medical records of potential participants in order to identify participants who may be eligible. CHLA has natural language processing algorithms that allows for rapid searches of electronic medical records to identify patients with specified criteria for recruitment into studies. Research staff will first discuss potential participants with the PI and treating physician before making contact. After the

initial recruitment process, a follow-up phone call will be made by a trained recruiter within one week to answer questions and solicit participation. Consent can be withdrawn from at any time. Potential participants will have the following carefully explained: (1) the investigational nature of the research; (2) the objectives of the research; (3) the procedures involved in the research; (4) any and all risks and discomforts due to participation; and (5) alternative options to participation that are currently available. Clarification questions will then be elicited from the potential participants and their parents/guardians. Only after the above will an IRB-approved document recording informed consent be signed as per institutional guidelines with a copy to be given to the participant and the original stored with the research record in a secure location. Data protection will be ensured by storing on an IS-approved university database on encrypted servers. All participants will be issued a study ID with data entered in de-identified manner. All databases will be password-protected with access only to the study team. All paper/documentation/research charts will be maintained in locked locations accessible only to the study team.

The IRB has strict regulations for the approach to and documenting of assent by minors and consent by their parents/guardians. Documentation of assent/consent is done via IRB-approved study-specific forms.

Setting

- All research procedures will be conducted in Children Hospital Los Angeles (Los Angeles, CA).
- The USC DORI may be utilized to collect study endpoints. At USC, study staff may complete study procedures during visits 1, 2, and 3 (refer to the Schedule of Study Procedure table). Additionally, research blood draws (baseline and post-meal samples will be assayed for glucose, insulin, c-peptide, GLP-1, GIP, PP at times -10, , 10, , 30, , 60, 90, 120, and 180 minutes after glucose) will be completed.

Resources Available

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Statistical analysis

Descriptive statistics and graphical depictions were utilized to assess the distribution of outcome variables. The primary outcome variables in this analysis were glucose, insulin, and c-peptide profiles and the main independent variables of interests were the time of meal consumption.

Mixed-effects linear regression model was used to assess changes in glucose, insulin, and c-peptide profile over the course of 180 minutes and the difference of first mealtime at 8:00AM, 12:00PM, and 4:00PM univariately. The difference of mealtime over the course of 180 minutes on glucose, insulin and c-peptide levels was assessed by including mealtime as an interaction term in the mixed-effects linear regression model. Due to the skewness of the distribution, differences in glucose, insulin, and c-peptide AUCs over 180 minutes and the insulinogenic index by mealtime were assessed on a logarithmic scale. Profiles that were significantly differ across meal timing were subjected to pairwise comparisons using the false discovery rate based on Simes' method. Changes in profiles were described using beta estimates (β) with its associated 95% confidence intervals and p-values. Pairwise comparisons were presented as percent changes with its associated 95% confidence intervals, unadjusted and adjusted p-values.

The secondary outcome variables included incretin levels and pancreatic hormones, which includes glucagon, pancreatic polypeptide, glucagon-like-peptide-1 (GLP-1), and glucose-dependent-insulinotropic polypeptide (GIP). Mixed-effects linear regression model was used to assess the changes in incretin levels and pancreatic hormones between pre- and post-intervention timepoints. Additionally, mealtime difference on the changes in incretin levels and pancreatic hormones was assessed by including an interaction term in the model, and these variables were analyzed on logarithmic scale. The distributions of incretin levels and pancreatic hormones were described in median and interquartile range by pre- and post- intervention timepoints. The paired difference of pre and post was described using median and 95% confidence intervals by mealtime. Statistical significance was set at 0.05 with two-sided test and all statistical computations were performed in Stata/SE 18.0 (StataCorp, College Station, TX).