Study Title: Effects of Low-calorie Sweetened Beverage Restriction in Youth with Type 1 Diabetes

NCT04385888

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List of Abbreviations

ACE-K	Acesulfame-potassium
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IRB	Institutional Review Board
LAR	Legally Authorized Representative
LCSB	Low-calorie sweetened beverage
LCS	Low-calorie sweetener
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SOP	Standard Operating Procedure
CTSI	Clinical and Translational Science Institute
COI	Conflicts of Interest
CITI	Collaborative Institutional Training Initiative

Protocol Summary

Title:	Effects of Low-calorie Sweetened Beverage Restriction in Youth with Type 1 Diabetes
Brief Summary:	This study will investigate whether low-calorie sweetened beverages (LCSBs) are helpful or harmful for preventing diabetes complications among children with Type 1 Diabetes (T1D).
Study Population:	The study population will consist of children (ages 5-14 years) with T1D, who report consumption of ≥ 12 oz. of a LCSB with sucralose (+/- ace-K) or aspartame+ace-K per day and their parent/guardian. The study population will also consist of up to 40 additional parent/guardians of children 5-14 with type 1 diabetes, who will participate in the qualitative interview about the impacts of the COVID-19 pandemic on their child's type 1 diabetes management.
Study Site:	Sheik Zayed Campus—Childhood and Adolescent Diabetes Program at Children's National.
Number of Participants:	60 Children (120 participants including parent/guardian) for RCT; up to 40 additional parents for COVID-19 interviews
Accrual Ceiling:	60 children (120 parents including parent/guardian) for RCT, 40 parents for COVID-19 interviews
Study Duration:	2 years
Subject Duration:	The total duration of subject participation for the RCT will be 14 weeks. The total duration of participation for the COVID-19 interviews will be approximately 30-45 minutes on a single day.
Objective(s):	To conduct a 12-week pilot intervention (involving a two-week run-in period prior to the intervention) to investigate the effects of LCSB consumption on children with T1D. To better understand the impacts of the COVID-19 pandemic on management of type 1 diabetes in childhood.
Methodology:	Pilot intervention study involving replacement of child LCSB consumption with unsweetened beverages (e.g. still or sparkling water) compared to control (continuation of

	usual LCSB consumption) and assessing glycemic variability using continuous glucose monitoring, as well as body composition and inflammatory cytokines before and after the intervention. Conduct of qualitative interviews about impacts of the COVID-19 pandemic on T1D management in a separate sample of parents (some of whom will have a child participating in the RCT).
Outcome Measures:	HbA1c, CRP, IL-6, TNF-α, LDL, HDL, triglycerides, and free fatty acids (FFA) measured in blood samples. Sucralose and ace-K concentrations in biological fluids, including urine. Visceral fat area from DXA or BodPod. Continuous blood glucose to assess glycemic variability, photo-assisted food records to assess adherence to LCSB restriction and any changes in diet throughout study, beverage log to assess adherence, satisfaction survey, Diabetes Self-Care Inventory (SCI), physical activity questionnaire (PROMIS), COVID-19 diabetes management questionnaire, Eating Attitudes (EAT) questionnaire, and an in-depth interview. Additional, in-depth interview about COVID-19 impacts on T1D management (for those who screen ineligible for full study andthose who participate in DRINK and are interested in also participating in this additional interview)
Study Intervention/ Procedures: **Statistical Analysis:**	Children will be randomly assigned to either replace their LCSB consumption with still or seltzer water or to continue their usual LCSB consumption for 12 weeks. Parents who participate in the COVID-19 interviews will undergo a brief qualitative interview with a trained interviewer. Univariate analyses and one-way ANOVA will be used to assess group differences in glycemic variability and other metabolic outcomes pre/post intervention. Bivariate associations will be examined using odds ratios, chi-squared, boxplots, mean differences, and ANOVA, as appropriate. Qualitative data collected during interviews will be transcribed verbatim and thematically coded.

SECTION 1 Key Roles

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SECTION 2 Introduction, Background Information, and Scientific Rationale

2.1 Background Information and Relevant Literature

Type 1 diabetes (T1D) is a lifelong metabolic disorder that affects 1 out of every 500 American children annually. Children diagnosed with T1D before the age of ten are at 11 times higher risk of cardiovascular disease, six times higher risk of stroke, and most notably, 31 times higher risk of coronary heart disease and myocardial infarction compared to similar-aged children without T1D. Furthermore, cardiometabolic perturbations, including abnormalities in circulating inflammatory cytokines, are observable in children with T1D despite their young age. As demonstrated in the SEARCH cohort, a common strategy for T1D management and prevention of cardiovascular complications is replacement of added sugars with low-calorie sweeteners (LCSs), which provide sweetness without calories and at a reduced glycemic load. However, effects of LCSs on glycemic control and cardiometabolic health are controversial. In cellular models, incubation of mesenchymal stem cells with sucralose at physiologic concentrations upregulates expression of genes involved in adipogenesis and inflammation. In rodents, prolonged sucralose consumption induces glucose intolerance, disturbs the gut microbiota, increases visceral adiposity, and promotes metabolic abnormalities. And, in humans, prospective cohort studies in adults without diabetes consistently report positive associations between LCS consumption, weight, metabolic syndrome, and cardiovascular disease (CVD). Furthermore, acute studies of LCS's effects in adults demonstrate elevated post-prandial glucose and insulin responses when LCSs are ingested prior to an oral glucose tolerance test (OGTT). Meanwhile, little is known about effects of LCSs in children, and low-calorie sweetened beverage (LCSB) consumption specifically in children with T1D is severely understudied. This is particularly concerning because hyperglycemia and inflammation play a key role in accelerated CVD onset in T1D and visceral adiposity is strongly linked to hypertension and dyslipidemia, further promoting CVD development. In this study, children with T1D are randomly assigned to either replace LCSBs with still or seltzer water for 12 weeks or continue usual LCSB consumption (control).

2.2 Scientific Rationale

The scientific premise of this proposal is that while use of LCSs in place of added sugars reduces the calorie and sugar content of foods and beverages, this replacement may not improve and may even paradoxically worsen glycemic variability and exacerbate cardiometabolic health among already at-risk children with T1D. Given that LCSB use, particularly LCSBs with sucralose or aspartame+acesulfame-potassium (ace-K), is widespread in children with T1D and even modest metabolic perturbations predict future CVD onset, there is tremendous significance in investigating the extent to

which replacement of usual LCSB consumption with still or seltzer water (hereafter referred to as LCS restriction) improves glycemic variability, visceral adiposity, lipid profiles, inflammation, and other CVD risk factors. The scientific premise for conducting qualitative interviews about the impacts of the COVID-19 pandemic on children's type 1 diabetes management is that this has not been in this population to date and has critical implications for the clinical care of children with type 1 diabetes.

Hypothesis

We hypothesize that participants will adhere to 12 weeks of LCSB restriction and will find the intervention acceptable. We also anticipate that 12 weeks will be enough time to observe changes in study outcomes. We hypothesize that an increase in the time in target glycemic range, a decrease in visceral adiposity and inflammatory cytokines, and an improvement in lipid profiles will be observed in those randomized to LCSB restriction vs. control.

Study population

The study population will consist of 60 children with T1D, ages 5-14 years old (and their parent(s)/guardian(s), n=120 including parent/guardian and child), who report habitual consumption of greater than or equal to 12 ounces of LCSBs with sucralose (+/- ace-K) or aspartame+ace-K per day. We will also a separate sample of up to 40 parents of children 5-14 years of age with type 1 diabetes to participate in interviews about the impacts of the COVID-19 pandemic on their child's type 1 diabetes management.

Study Interventions

This study includes an intervention where children are randomly assigned to either replace LCSBs with still or seltzer water or to continue usual LCSB consumption (control) in order to determine whether LCSB restriction impacts glycemic variaiblity and cardiometabolic risk factors in 5-14-year-old children with T1D.

2.3 Potential Risks

It is possible that other participants and individuals external to the study may find out that a participant took part in the research, in which case, information derived may be conveyed to others external to the group.

Blood Draws: Although blood draws are a standard medical procedure, there is a risk of discomfort at the site of the needle entry or bruising at the site. There is also a remote risk of fainting or local infection associated with drawing blood. To address this, only skilled and well-trained phlebotomists will carry out these procedures.

Continuous glucose monitoring (CGM): The use of CGM for measurement of glycemic variability and 'time in range' may be challenging for some children and their parents. Although CGM is commonly used in children with T1D, some children may be fearful of the insertion or embarrassed to share their blood glucose levels. We will work with the child and parent to ensure the child is comfortable. We will also discontinue CGM if this becomes painful or problematic to the child or parent.

Dual energy X-ray absorptiometry (DXA): This instrument is used to measure body fat using low dose x-ray. This scan does give a small amount of radiation. Using the standard way of describing radiation exposure, subjects will receive an effective dose of less than one thousandth of one rem from each DXA scan. By comparison, the average person in the United States receives approximately this much radiation every day from natural background sources, such as the sun and from radioactive materials that are found naturally in the earth's air and soil.

Spot urine sample: We are not aware of any known risks from providing a spot urine sample. However, collecting the sample may be uncomfortable, burdensome, or embarrassing for some individuals. We have minimized this risk by having participants collect the urine sample in a private space and de-identifying the data collected to ensure that urine samples are not directly linked to the subject's identity.

BodPod: Children may experience ear popping while in the BodPod and some children may also feel claustrophobic or be otherwise nervous while in the BodPod. We have minimized this risk by excluding any child with acute illness from study participation (including those with ear or sinus infections) and working with the child and parent to ensure the child is comfortable prior to the BodPod. Furthermore, only trained staff in the Clinical Research Unit will conduct the BodPod assessments.

Qualitative interviews: Completing interviews may be uncomfortable, burdensome, or embarrassing for some individuals. We have minimized this risk by de-identifying the data collected to ensure that responses to the questions are not directly linked to the subject's identity. Participants will not have to answer questions if they do not wish to do so.

Food Records: We are not aware of any known risks from completing food records. However, completing food records may be uncomfortable, burdensome, or embarrassing for some individuals. We have minimized this risk by de-identifying the data collected to ensure that beverage logs are not directly linked to the subject's identity.

Questionnaires: We are not aware of any known risks from completing questionnaires. However, completing the EAT assessment, satisfaction survey, PROMIS physical activity questionnaire, or COVID-19 diabetes management questionnaire may be uncomfortable, burdensome, or embarrassing for some individuals. We have minimized this risk by de-identifying the data collected to ensure that questionnaire responses are not directly linked to the subject's identity.

Beverage logs: We are not aware of any known risks from completing beverage logs. However, completing beverage logs may be uncomfortable, burdensome, or embarrassing for some individuals. We have minimized this risk by asking participants to submit beverage logs via text message or email and de-identifying the data collected to ensure that beverage logs are not directly linked to the subject's identity.

We do not anticipate any long-term risks to participants from participation in this study. However, there is always the possibility of risks currently unknown and the potential breach of privacy. To minimize the risk of breach of confidentiality, all data will be immediately de-identified following collection. Data will initially be associated with an alphanumeric code and a copy of the key to this code will be kept in a locked filing cabinet. All computers used for analysis of this data are password protected and kept in locked offices and/or laboratories.

All participants will be made aware that study participation is entirely voluntary and will have the opportunity to choose not to participate. Subjects will have the opportunity to drop out of the study at any time. The potential benefits outweigh the risks because the study is minimal risk and procedures do not exceed the risks encountered in routine medical procedures. Furthermore, what we learn about LCSB consumption among children with T1D will be critical to informing the design of future interventions.

2.4 Potential Benefits

There are no direct benefits to the participants of the study. However, the potential public health benefits of this study are of substantial magnitude. A common strategy for diabetes management and prevention of cardiovascular complications is replacement of added sugars with LCSs, which provide sweetness at a reduced glycemic load. However, the effects of LCS on glycemic control and cardiometabolic health are controversial and little is known about the effects of LCS consumption in youth, specifically in those with T1D.

This study will determine the effect of chronic LCSB use among children with T1D on glycemic control and cardiometabolic health. Given that modest metabolic perturbations predict future CVD onset, there is tremendous significance in investigating the extent to which replacement of LCSB consumption improves glycemia, visceral adiposity, lipid profiles, inflammation, and other CVD risk factors.

SECTION 3 Objectives and Endpoints

3.1 Primary Objective(s)

To examine the effects of replacement of usual LCSB consumption with unsweetened still or seltzer water for 12 weeks on glycemic variability using continuous glucose monitoring (CGM) compared to control (continuation of usual LCSB consumption).

3.2 Secondary Objective(s)

To examine the effects of replacement of usual LCSB consumption with unsweetened still or seltzer water for 12 weeks on visceral adiposity, lipid profiles, and systemic inflammation compared to control (continuation of usual LCSB consumption). To examine the impacts of the COVID-19 pandemic on pediatric type 1 diabetes management.

3.3 Primary Outcome Measure(s)

Glycemic variability and HbA1c

3.4 Secondary Outcome Measure(s)

Visceral adiposity, lipid profiles, and systemic inflammation, impacts of COVID-19 pandemic on type 1 diabetes management from qualitative interviews

SECTION 4 STUDY DESIGN

Eligible volunteers for the RCT will be identified prior to or during their routine clinical visit in the Childhood and Adolescent Diabetes Program (CADP) at Children's National Hospital and will be provided with study information by their physician or a member of the research team either via mail (if prior to visit), email, phone and/or or in person (recruitment letter and flyer enclosed), or will be reached out to via social media and word of mouth. The parent will complete a brief phone screening to determine initial study eligibility. If their child is eligible for the RCT, they will be scheduled for a screening/enrollment visit via Zoom Telehealth and will also have the option of participating in a separate interview about the impacts of the COVID-19 pandemic on their child's diabetes management (separate consent form, survey, and interview guide enclosed).

If their child is ineligible for the RCT, the parent will be invited to participate in the qualitative interview about impacts of the COVID-19 pandemic on T1D management, which will be scheduled at a time convenient for the parent and will be administered remotely via Zoom Telehealth. All parents of children 5-14 years old with T1D, and whose child has had T1D for at least one year, will be eligible to participate in the Zoom Telehealth interview. The interview will be conducted at a date/time convenient for the parent and will take place remotely via Zoom Telehealth. Participation in the interview about impacts of the COVID-19 pandemic on diabetes management will involve 1) providing informed consent via Redcap 2) answering questions about the parent's sex and race/ethnicity and the child's age and duration of diabetes, 3) answering brief survey questions about diabetes management during the COVID-19 pandemic and whether they are vaccinated (enclosed), and 4) participating in a 20-30 qualitative interview about the impacts of COVID-19 on their child's diabetes management, conducted by Dr. Meni.

For those determined to be initially eligible for the RCT, the screening/enrollment procedures will proceed as follows: after obtaining informed consent (from the parent/guardian(s)) and assent (from the child) the research team will guide the participant through the SCI, PROMIS physical activity, COVID-19 diabetes management questionnaire, and EAT assessments, and a Dexcom™ sensor will be placed on the child's abdomen. The sensor will remain in place for 7-10 days. The parent will then replace the sensor (provided by study team) to allow for 14 days of wear-time, until removal at the baseline visit. Participants will be randomly placed in one of two groups. If the participant is already using CGM, they will have the option to have this first study meeting remotely via Zoom Telehealth. If the participant attends the visit via Zoom Telehealth, the participant and their parent will provide informed consent using the recently approved RedCap e-consent platform. At the end of the screening, participants

will be provided with low-calorie sweetened beverages, matched as closely as possible to the brands that they usually consume, to encourage continuation of usual beverage intake between until the baseline visit. During the week prior to the baseline visit (Week 0), they will be instructed to continue their usual dietary habits and complete an online photo-assisted 7-day food record (instructions enclosed), with parental assistance. While we recognize that parents are unlikely to accurately report all of their child's intake because they are not always with them (e.g., during school), dietary data for children under 14 years are most accurately reported when provided by the parent or child and parent combined.

Eligible participants will be scheduled for a baseline visit in the Clinical Research Unit (CRU) at CNMC. One group will replace their usual LCSB consumption with unsweetened still or seltzer water (LCSB restriction) for 12 weeks. The other group will continue their usual LCSB use (control) for 12 weeks. The baseline visit will take place in the CRU, where height and weight will be measured, a spot urine sample will be collected (for measurements of sucralose and ace-k concentrations), and a blood draw will be performed. Participants will be provided with a copy of their laboratory (blood) results from the baseline visit and will be encouraged to discuss them with their physician. Participants will have *the DEXCOM G6™ sensor removed and* CGM data downloaded*.* In a subset of participants (n=30), a DXA scan or BodPod will also be performed, as R21 budget constraints preclude DXA or BodPod on all 60 subjects.

Subjects will be randomized to either: 1) LCSB restriction or 2) continuation of usual LCSB intake (control). All participants (both LCSB restriction group and control group) and their parent(s) or guardian(s) will undergo a brief, 20-minute orientation where the PI and study dietitian will provide an introduction to the study, instructions on completing food records*, education on* CGM *and placement of the DEXCOM G6™ sensor*, and counseling on healthy eating strategies. For children not already using CGM, parents will also receive 3 additional sensors (replacement in Week 1, placement in Week 11, and replacement in Week 12 for 14 days wear time), written instructions and additional resources (YouTube video) for placing (or replacing) the glucose sensors. Participants in the intervention group will be counseled on avoiding LCSBs. Participants and their parent will also be given a brochure (enclosed) on avoiding LCSs to take home which will include a list of specific foods and beverages containing LCSs to avoid during the study. Parents will also receive information on strategies to encourage study adherence (enclosed). Participants in the control group will be counseled on healthy eating as those in the intervention group (in accordance with standard dietary guidance for T1D management), with the exception of information and resources for avoiding LCSBs. Participants in both groups will be provided with sample beverages (LCSBs or unsweetened still and seltzer water), per their randomization.

In both groups, text messages will be sent to parents 3 times per week with reminders that their child should avoid LCSBs or continue usual intake, per randomization. Adherence will be monitored through daily text message beverage logs completed by the parent and photo-assisted food records with parent assistance in Weeks 0, 6, and 12, as well as spot-urine samples (to measure sucralose and ace-K concentrations

(baseline, week 6, and follow-up visit). During Week 6, participants and their parent will attend a mid-intervention telemedicine booster visit, during which the PI and/or study RA will reinforce the intervention and remind participants to mail back a spot urine sample using materials provided by the study team at baseline. Participants will be reminded of the study instructions, including the importance of inserting the DEXCOM G6 sensor (provided by study team) at the beginning of Week 11. Parents will receive a phone reminder to insert the sensor at the end of Week 10 and a second call early in Week 11 to ensure that the sensor has been placed and will be replaced after 7-10 days to allow for 14 days of wear time prior to the follow-up visit. Depending on randomization assignment, guidance to avoid LCSBs or continue usual diet will be reinforced. Participants will be reminded to store their CGM data for weeks 11 and 12. All participants and their parent will have the opportunity to chat in real-time with the study team about questions or concerns.

At the end of Week 12, participants and their parent will return for follow-up in the CRU. Participants will have their DEXCOM G6™ sensor removed and CGM data downloaded using DEXCOM Clarity™ software. Height and weight will be measured and a second blood draw performed. Participants will again be provided with a copy of their laboratory (blood) results and encouraged to discuss them with their physician. Those who had an DXA at the baseline visit (n=30) will undergo a second DXA or BodPod scan after which these participants and their parent will receive a consultation on the DXA or BodPod results. Those randomized to the intervention will be purposefully sampled and asked to complete a ~20-minute qualitative interview and ~5 minute satisfaction survey, together with their parent, about their study experience and the challenges of LCSB restriction. This will allow us to collect information from individuals with a range of perspectives and varying degrees of intervention adherence. Sampling will continue until saturation of ideas is reached or until all subjects in the intervention group are invited to be interviewed.

SECTION 5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 Study Population, Recruitment and Retention

Potentially eligible participants will be identified through CNMC's medical records prior to routine clinic visits. The study will also be advertised to T1D patients in the CADP through a trained research assistant (RA) in clinic waiting rooms at the time of their routine clinic visits. If a participant expresses interest and meets eligibility criteria, a screening visit will be scheduled for directly before or after the patient's routinely scheduled clinic visit. Participants will also be recruited via word of mouth and social media, recruiting children with T1D and their parents willing to travel to Children's National for the in person components. During the screening visit the child and their parent/guardian (referred to as parent) will complete a LCSB screener questionnaire and brief eligibility checklist, including the Eating Attitude Test (EAT) administered by the RA to determine initial eligibility (which will be confirmed by Dr. Cogen). Eligible participants and their parent will provide assent and consent, respectively.

To encourage adherence and retention, we will provide $340 for study participation (this includes $120 for the baseline visit, $120 for the follow-up visit, $30 for booster session, $40 for 4 food records, $30 for the screening visit). The subset of participants who undergo DXA or BodPod will receive an additional $50 per visit, for a total of up to $440 compensation for study participation. Additional compensation will be provided to cover the cost of parking (parking voucher) or transportation (equivalent up to $10) to Children's for study visits. We will also encourage retention by maintaining regular contact with study participants through text message/email reminders and the 6-week telemedicine booster session.

5.2 Inclusion Criteria (RCT)

- Child is between 5 to 14 years old
- Child consumes ≥ 12 oz. of a LCSB with sucralose (+/- ace-K) or aspartame+ace-K per day
- Child has had a diagnosis of T1D for at least one year
- Parent/guardian has reliable phone and internet access
- Parent/guardian and child both speak English

Inclusion Criteria (COVID-19 interviews)

- Parent of child with T1D between 5 to 14 years old
- Parent of child who has had a diagnosis of T1D for at least one year
- Parent/guardian has reliable phone and internet access
- Parent/guardian speaks English fluently
- Parent of child enrolled in the Childhood and Adolescent Diabetes Program (CADP) at Children's National

5.3 Exclusion Criteria (RCT)

- Child consumes foods with low-calorie sweeteners (e.g., sugar-free ice cream, light yogurt, sugar-free oatmeal) more than 3 times per week
- Child consumes condiments with low-calorie sweeteners (e.g., sugar-free pancake syrup, sugar-free jam, reduced sugar ketchup) more than 3 times per week
- Child has a poorly managed chronic disease other than T1D, acute illness, current or prior eating disorder as assessed by the EAT or is taking medications other than insulin

Exclusion Criteria (COVID-19 interviews)

- **Not applicable.**

5.4 Inclusion of Children

We will study children 5-14 years, rather than older adolescents, because children of this age are more often monitored by their parents than older children, enabling us to more effectively engage parents to maximize intervention adherence. Children 4 years

and younger will be excluded because LCSB consumption is relatively low in this age group.

5.5 Recruitment

The study (both the RCT and the COVID-19 interviews) will be advertised to the clinic patient populations and their parents through on-site recruitment in clinic waiting rooms and through searching CNMC medical records prior to their routine clinic visits, as well as via social media, list servs, and word of mouth. Potentially eligible patients will be provided with study information by in-person, via email, postal mail or by phone (recruitment letter for RCT enclosed).

For the COVID-19 interviews, if a parent expresses interest and meets eligibility criteria, they will be scheduled for a Zoom Telehealth interview at a day/time convenient for them.

For the RCT, if a participant expresses interest and meets initial eligibility criteria (assessed in person or via phone), a screening visit will be scheduled immediately before or after the patient's upcoming clinic visit to ensure minimal disturbance to clinic flow. If the patient is already using CGM, the participant will have the option to complete this screening visit remotely via Zoom Telehealth rather than in person. Given the large volume of children served by the participating sites, we do not anticipate any problems recruiting and retaining a diverse sample of children. If the potential participant has questions that the research assistant (RA)/clinical study coordinator (CRC) cannot answer, the RA/CRC will immediately contact Dr. Streisand.

5.6 Retention

We will maximize retention by maintaining regular contact with RCT participants through text message/email reminders throughout the study. This will help us to develop rapport with the subjects. In addition, we will provide a total of $340 for participating in the study. The subset of participants who undergo DXA or BodPod will receive an additional $50 per visit, $440 total. Compensation will be provided using the Forte payment system (analogous to ClinCard) at GW. For parents who participate in the COVID-19 interviews, compensation of $50 will be provided and will be provided as a gift card.

5.7 End of Participation Criteria and Procedures

Participants are free to withdraw from participation in the study at any time upon request. An investigator may terminate study participation if:

- The participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation.
- The participant is unwilling or unable to comply with the study procedures.

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification documenting the reason for study suspension or termination will be provided by the suspending or terminating party to participants, site investigators, the funding agency and regulatory authorities (e.g., OHRP). If the study is

prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

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

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient study team or site participant compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility

The study may resume once any concerns about safety, protocol compliance, data quality or funding are addressed and satisfy the sponsor, IRB and OHRP.

SECTION 6 STUDY PROCEDURES

6.1 Informed Consent/Assent

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study. It continues throughout the individual's study participation. Consent and assent forms will be IRB-approved. The parent and participant will be asked to read and review the consent and assent document(s), respectively. Extensive discussion of risks and possible benefits of participation will be provided to the participants and their families. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal lay explanation of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants and their parent/guardian will have the opportunity to carefully review the consent and assent form and to ask questions prior to signing. Documentation of parent informed consent (written or using RedCap e-consent platform) and child assent is required for all participants prior to enrolling in the study of undergoing any procedures.

The parent and their child will sign the informed consent and assent, respectively, prior to beginning any study procedures. The participants may withdraw consent/assent at any time throughout the course of the study. A copy of the informed consent document(s) will be given to the participant/LAR for their records. The rights and welfare of the participants will be protected by emphasizing that the quality of their medical care will not be adversely affected if they decline to participate in this study.

If the participant has questions that the research assistant (RA)/clinical study coordinator (CRC) cannot answer, the RA/CRC will immediately contact Dr. Streisand to provide clarification. The above described process for obtaining informed consent will also apply for obtaining consent from parents participating in the separate, qualitative interview about impacts of the COVID-19 pandemic on T1D management. No assent is needed for the qualitative interviews pertaining to impacts of the COVID-19 pandemic on T1D management because only the parent will participate.

6.2 Screening Process

Subjects will be screened in-person or via Zoom Telehealth or phone before or after patient's upcoming clinic visit. Participants will be asked about each of the inclusion/exclusion criteria using the eligibility screener questionnaire, and the EAT, and will be asked to answer a brief prompt ("The COVID-19 pandemic has impacted management of my/my child's type 1 diabetes by…") about the impact of the COVID-19 pandemic on diabetes management. Any eligible participant will be invited to participate until our total sample size is reached.

6.3 Study Interventions and Follow-Up

At the end of Week 12, participants and their parent will return for follow-up in the CRU. Participants will have their *DEXCOM G6™ sensor removed and* CGM data downloaded using DEXCOM Clarity™ software. Height and weight will be measured and another urine sample and blood draw performed. Those who had a DXA scan or BodPod at the baseline visit (n=30) will undergo a second DXA scan or BodPod, after which these participants and their parent will receive a consultation on the results. Those randomized to the intervention will be purposefully sampled and asked to complete a ~20-minute qualitative interview and ~5 minute satisfaction survey, together with their parent, about their study experience and the challenges of LCSB restriction. This will allow us to collect information from individuals with a range of perspectives and varying degrees of intervention adherence. Sampling will continue until saturation of ideas is reached or until all subjects in the intervention group are invited to be interviewed. There is no follow-up for participants in the COVID-19 interviews.

6.4 Description of Study Procedures/Evaluations

We propose a two-arm randomized controlled trial (RCT) during which 60 children with T1D will either replace usual LCSB consumption with unsweetened still or seltzer water (LCSB restriction) or continue usual LCSB use (control) for 12 weeks. This will provide ample time to evaluate intervention feasibility and acceptability, while enabling us to generate preliminary evidence of whether LCSB restriction may improve cardiometabolic health.

Eligible participants will be scheduled for a baseline visit in the Clinical Research Unit (CRU) at CNMC. During the week prior, they will be instructed to continue usual dietary habits and complete an online photo-assisted 7-day food record, with parental assistance. While we recognize that parents are unlikely to accurately report all of their child's intake because they are not always with them (e.g., during school), dietary data for children under 14 years are most accurately reported when provided by the parent or child and parent combined.

The baseline visit will take place in the CRU, where height and weight will be measured, a spot urine sample will be collected, and a blood draw will be performed. Participants will have the DEXCOM G6™ sensor removed and their CGM data downloaded. In a subset of participants (n=30) a DXA scan or BodPod will also be performed, as R21 budget constraints preclude DXA or BodPod on all 60 subjects. Subjects will be

randomized **(Section C.6)** to 1) LCSB restriction or 2) usual LCSB intake (control). All participants (both control and LCSB restriction) and their parent will undergo a brief, 20-minute orientation where the PI and study dietitian will provide an introduction to the study, instructions on completing food records*, additional education on* CGM *and placement of the DEXCOM G6™ sensor*, and counseling on healthy eating strategies. *Parents will also receive 3 additional sensors (replacement in Week 1, placement in Week 11, and replacement in Week 12 for 14 days wear time), written instructions and additional resources (YouTube video) for placing (or replacing) the glucose sensors.* Those in both groups will be *given sample study beverages at baseline (several varieties of still and seltzer water for intervention group or LCSBs matched as possible to those typically consumed in the control group) to encourage adherence. Those in the intervention group will be* counseled on avoiding *LCSB. Participants* and *their parent* will *also* be given a brochure on avoiding LCSBs to take home, which will include a list of specific foods and beverages containing LCSBs to avoid during the study. Participants in the control group will be counseled by Dr. Meni or a research assistant, and will receive identical counseling on healthy eating as those in the intervention group (in accordance with standard dietary guidance for T1D management), with the exception of information and resources for avoiding LCSB.

In both groups, text messages will be sent to parents 3X/week with reminders that their child should avoid LCSBs or continue usual intake, per randomization. Adherence will be monitored through collection of spot-urine samples for measurement of sucralose and ace-K concentrations (baseline, week 6, week 12), daily text message beverage logs completed by the parent, and photo-assisted food records with parent assistance in Weeks 0, 6, and 12. During Week 6, participants and their parent will attend a mid-intervention telemedicine booster visit, during which the PI and/or study RA will reinforce the intervention and remind participants to mail back a spot urine sample using materials provided by the study team at baseline (instructions enclosed). Participants will be reminded of the study instructions, including the importance of inserting the DEXCOM G6 sensor (provided by study team) at the beginning of Week 11. Parents will receive a phone reminder to insert the sensor at the end of Week 10 and a second call early in Week 11 to ensure that the sensor has been placed and will be replaced after 7-10 days to allow for 14 days of wear time prior to the follow-up visit. Depending on randomization assignment, guidance to avoid LCSBs or continue usual diet will be reinforced. All participants and their parent will have the opportunity to chat in real-time with the study team about questions or concerns.

At the end of Week 12, participants and their parent will return for follow-up in the CRU. Participants will have their DEXCOM G6™ sensor removed and CGM data downloaded using DEXCOM Clarity™ software. Height and weight will be measured and a urine sample and blood draw performed. Those who had a DXA or BodPod at the baseline visit (n=30) will undergo a second DXA or BodPod, after which these participants and their parent will receive a consultation on the DXA or BodPod results. Those randomized to the intervention will be purposefully sampled and asked to complete a ~20-minute qualitative interview and ~5 minute satisfaction survey, together with their parent, about their study experience and the challenges of LCSB restriction. This will

allow us to collect information from individuals with a range of perspectives and varying degrees of intervention adherence. Sampling will continue until saturation of ideas is reached or until all subjects in the intervention group are invited to be interviewed. Study procedures are detailed below.

Table 1. Timing of Study Procedures

Time-point	Location	Assessments	Procedures
Interview about COVID-19 impacts on T1D management (separate from rest of trial)	Home	Qualitative interview	Consent, questionnaire, qualitative interview
Screening Visit	Clinic or Home	Eligibility Determination, Enrollment, Questionnaires	Screener, consent, assent, EAT, self-care (SCI), COVID-19 questionnaire, PROMIS questionnaire
Week 0	Home	Diet assessment, glycemic variability	Food record, *CGM,* beverage log
Baseline Visit	CRU	Glycemic control (HbA1c), lipids, inflammation, visceral adiposity, anthropometrics	Height/weight, spot urine, blood draw, CGM download, DXA or BodPod (subset)
Weeks 1-5	Home	Adherence	Food record, beverage log
Week 6	Home	Diet assessment, adherence, intervention booster	Food record, beverage log, spot urine
Week 7-10	Home	Adherence	Beverage log
Week 11-12	Home	Diet assessment, glycemic variability, adherence	Food record, *CGM,* beverage log
Follow-up Visit	CRU	Glycemic control (HbA1c), lipids, inflammation, visceral adiposity, anthropometrics, acceptability, feasibility	Height/weight, spot urine, blood draw, SCI, satisfaction survey, qualitative interview, CGM download (for weeks 11 and 12), DXA or BodPod

Laboratory analyses: All blood assays will be run by LabCorps. Urine analyses will be performed by the Clinical Mass Spectrometry Laboratory at the National Institutes of Health. Blood and urine samples will be shipped by certified personnel in the CTSI-CN PCI at Children's National.

Additional blood and urine specimens will be stored in the CTSI-CN PCI at Children's National for up to three years after completion of data collection for future analyses. All samples will be stored deidentified. Depending on the future analyses performed, deidentified samples may be shipped to external commercial or academic laboratories in the future.

6.5 Study Team Training and Intervention Reliability

All study team members have completed required human subjects training and have undergone protocol-specific training with respect to the intervention and all study procedures.

SECTION 7 STATISTICAL CONSIDERATIONS AND ANALYSIS

Our primary outcome is the difference in change in glycemic variability in those randomized to LCSB restriction compared to controls, calculated as difference in average daily TIR in Weeks 11 and 12 compared to Weeks -1 and 0. Because no prior study has assessed glycemic variability before and after LCSB restriction, we have powered our study using changes before and after LCS consumption. Suez et al., reported a 1564 ± 1852 $(mg \cdot dL \cdot 120\ mins)^{-1}$ increase in glucose AUC post-LCS versus baseline, in 7 volunteers consuming saccharin (equivalent to 4-5 diet sodas per day) for 1 week. Using a similar effect size, 13 subjects per group will provide 80% power to detect differences in glycemic control before and after LCS restriction versus control (G*Power; V3). Because 60% (4 of 7) of participants were considered LCS-responders by Suez, we will randomize 20 participants to the intervention and control, respectively (n=40). 48 subjects (24 per group) will account for 20% attrition. However, because prior studies conducted by Drs. Streisand and Cogen in this patient population have generated 70-80% usable CGM data, we will conservatively enroll 60 subjects (n=30 per group). We will use 1:1 block randomization stratified by LCSB type (sucralose or aspartame + ace-K) to lay the groundwork for evaluating potential differences in effects of sucralose versus aspartame in future studies.

Univariate analyses will be performed using an intention to treat approach. The maximum number of days of glycemic data will be used without imputations for missingness. Bivariate associations will be examined using cross tabulations, odds ratios, and chi-squared tests; boxplots, mean differences, and ANOVA, and correlations, as appropriate. We will identify and validate outliers and non-parametric tests will be used in the case of non-normal data. By establishing strengths of crude associations and highlighting irregular data, descriptive analyses will be a reference for more complex models. Changes in glycemic variability and other metabolic outcomes pre/post intervention will be compared between the groups using ANOVA. Hypothesis

testing will be two-sided with a type I error of 0.05. Analyses will be summarized using regression estimates, p-values, and 95% CI and performed using SAS 9.4.

Study recruitment, enrollment, and rates of data completion will be used to examine feasibility. To assess acceptability, attrition rates will be examined, and qualitative interviews will be conducted using 8 open-ended questions to probe for participant acceptability, feasibility, and situations where adherence was most challenging. Adherence to beverage assignments will be operationalized as any consumption of LCSBs in violation of the study protocol. Data collected during qualitative interviews (for both interviews at end of full trial and for the separate interview on COVID-19 impacts on T1D management) will be transcribed and coded using standard methods and analyzed using content analysis. The codebook will include descriptive categories based on a priori research questions as well as data-driven categories to identify emergent themes. Streisand, and a trained RA will code the first 3 transcripts together to develop a preliminary codebook. The RA will independently code the remaining transcripts and modify the codebook as themes arise. The team will discuss any discrepancies in coding, after which Dr. Streisand will make the final determination.

SECTION 8 DATA QUALITY AND OVERSIGHT

8.1 Study Team Quality Assurance and Quality Control

We have developed a quality assurance and data management plan to ensure that compliance with our protocol is maintained throughout the study. This will facilitate the collection of reliable data, leading to credible research findings with the potential to advance the field. The plan outlined below will also ensure that the safety and rights of our human subjects are protected.

Quality control (QC) and quality assurance (QA) will be ongoing throughout the study and will primarily by the responsibility of the Principal Investigator Dr. Streisand, who will also be responsible for quality management, including oversight of the informed consent process. Dr. Streisand will oversee the day-to-day quality control activities (e.g., potential errors in data entry).

In addition to the required Human Subjects Training, all study team members will receive additional training on all aspects of the protocol. Training will be administered by Dr. Streisand. This will include specific instruction on the following components:

1. Study Objectives: All study team members will be required to read the study protocol in its entirely and have a thorough understanding of the rationale and significance of the study. All study team members will be required to explain the study outcomes of interest and how they are related to the study objectives set forth in the protocol.
2. Inclusion/Exclusion Criteria: All study team members will be asked to make eligibility determinations using test screener questionnaires.
3. Protocol Deviations: All study members will be provided with a detailed SOP for detection and reporting of protocol deviations.

SECTION 9 ETHICAL CONSIDERATIONS

9.1 Ethical Standard

The study team will ensure that this study is conducted in full conformity with the Regulations for the Protection of Human Subjects of Research codified in 45 Part 46 of the Code of Federal Regulations, Children's National Policies and Procedures and Good Clinical Practices.

9.2 Institutional Review Board (IRB)

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Children's National IRB for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is consented. Any change to the protocol, consent, recruitment materials and participant information sheets or letters will require IRB approval before implementation and use. The IRB will determine whether previously consented participants need to be re-consented and whether consent of more than one parent is required for minors. The IRB will be notified of study team updates via an amendment. Other study events (e.g., protocol deviations, data monitoring reports) will be submitted per the Children's National IRB Reportable Events Module.

9.3 Maintaining Subject Privacy

All study procedures will take place in a private room to ensure the participant's privacy. The study participant's contact information will be securely stored at each study site for internal use during the study. At the end of the study, all research records will be stored in a secure location for the time period dictated by the sponsor and institutional regulations. The research data will not include the participant's identifying information. PHI including name, phone number, and email address will be collected for the clinical pilot study; however, all data will be de-identified and PHI will not be disclosed. The study data entry and study management systems used by research staff will be secured and password protected.

9.4 Maintaining Study Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, the sponsor and their agents. This confidentiality is extended to cover information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The sponsor representatives and regulatory authorities (e.g., IRB, OHRP) may inspect all documents and records required to be maintained by the investigator. The clinical study site will permit access to such records.

9.5 Certificate of Confidentiality

No Certificate of Confidentiality will be obtained for this study.

9.6 Study Support and Conflicts of Interest

REDCap® support is provided by The Clinical and Translational Science Institute (CTSI) at Children's National. All key study personnel will follow the Human Research Protections Program Investigator, Study Staff, and Family Member Conflicts of Interest (COI) Policy.

SECTION 10 DATA QUALITY AND OVERSIGHT

10.1 Data Management Responsibilities

All data will be entered and managed using RedCap, a password-protected data management system. Data will be entered by trained research assistants and Dr. Streisand will check RedCap weekly during periods of active data collection to ensure that data entry is correct and up to date.

10.2 Data Capture Methods

Data collection is the responsibility of the trial staff. The PI is responsible for ensuring the accuracy, completeness, legibility, timeliness and completeness of the data reported. Source data include all responses to study questionnaires and dietary recalls.

10.3 Study Record Retention Policy

Dr. Streisand will keep all study records in a locked cabinet for 5 years after enrollment of the last participant.

SECTION 11 ETHICAL CONSIDERATIONS

All study investigators will be co-authors on any publications resulting from this research. No subjects will be identified by name in any of the published literature. All study results will be published in aggregate and not attributable to individual participants.

SECTION 12 *APPENDIX*

Eligibility Screener Questionnaire

All answers must be 'YES' for study eligibility.

- Has your child been diagnosed with Type 1 Diabetes (T1D)?
- Has your child had a diagnosis of T1D for at least one year?
- Is your child between 5 to 14 years old?
- Does your child consume at least 12 ounces of a low-calorie sweetened beverage on most days (e.g., diet soda, light fruit juice, reduced sugar sports drink, diet iced tea*?*
    - If so, what is the brand of the beverage and does it contain sucralose and/or aspartame+ace-K?

- Do you have reliable phone and internet access?
- Do you and your child both speak English?
- Are you enrolled in the Childhood and Adolescent Diabetes Program at Children's National?

All answers must be 'NO' for study eligibility.

- Does your child consume foods with low-calorie sweeteners (e.g., sugar-free ice cream, light yogurt, sugar-free oatmeal) more than 3 times per week?
- Does your child consume condiments with low-calorie sweeteners (e.g., sugar-free pancake syrup, sugar-free jam, reduced sugar ketchup) more than 3 times per week?
- Are you currently participating in any other studies?