Title: Effect of Reducing Added Sugar Intake, By Substituting Sparkling Water, on Glycemia in Adolescents at Risk for Type 2 Diabetes

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1.0 Background & Rationale

Importance of studying added sugars in adolescents. Research that leads to nutritional strategies to prevent T2D in adolescents is an urgent pursuit [1]. One particular contributing factor to the growth of T2D and prediabetes in adolescents is the increased consumption of added sugars. Adolescents in the U.S. have high intakes of added sugars [2-6] which could have poor health implications. Added sugars are found in sugar sweetened beverages and desserts and snacks, top sources of energy for adolescents, and calories from added sugars contribute to 16% of the total energy intake [7, 8]. Levels are well above recommendations from leading scientific organizations, which suggest limiting added sugars to less than 10% of energy [9-12]. However, it is not clear whether sugar reduction to less than 10% of energy is palatable for an adolescent population that prefers sweetened foods and beverages. Moreover, uncertainty about the evidence linking added sugar consumption to risk of disease has made policies that seek to reduce dietary intake of added sugar controversial [13]. The controversy impairs large-scale implementation of recommendations to reduce intake of added sugars by individuals, clinicians, policy makers and industry stakeholders [14].

Evidence linking added sugars and risk of type 2 diabetes and cardiovascular disease lacks information on adolescents. Many epidemiological studies in adults [15-17], but not all [18] show the concept that after controlling for energy intake, high consumption of sugar-sweetened foods is a risk factor for T2D. Epidemiological studies also associate high consumption of added sugars, particularly from sugar sweetened beverages (SSB), with increased adiposity (including central adiposity) and dyslipidemia. These studies formed the basis of recommendations to limit added sugar consumption from the American Heart Organization [9]. The data in youth is limited. Two small randomized studies compared the effects of glucose versus fructose beverages on insulin sensitivity in adolescents and findings were mixed [19, 20]. One longitudinal cohort study showed that higher consumption of added sugars, from SSB, was associated with hyperglycemia and impaired insulin sensitivity in youth aged 8 – 12 years [21]. One study showed that high amounts of added sugars may induce insulin resistance and beta-cell dysfunction and such effects can occur independent of adiposity [22]. ***The literature lacks evidence linking high consumption of added sugars to risk of developing youth-onset T2D.***

Substituting Flavored Sparkling Water for Sugar Sweetened Beverages. Instead of sugar, food manufacturers typically sweeten foods beverages using low-calorie or artificial sweeteners. “Diet” versions of SSB have been available for several decades and are widely used by adults and children [23]. However the safety of low calorie sweeteners is controversial [24] and many in the scientific and lay communities do not promote their use as a substitute for SSB. We propose that an alternative to SSB may be found in sparkling waters (carbonated water with flavoring, completely unsweetened), which are commercially available in soda-like flavors like cola or Dr. Pepper™. The rationale for our proposal is the urgent need for an effective strategy to combat adolescent T2D which we will address by demonstrating the benefit of an intervention to reduce added sugars. The objective of the study is to demonstrate whether substituting sparkling water for SSB has benefits for in vivo physiological measures of glycemia while maintaining flavor acceptability to adolescents who are at risk for diabetes.

2.0 Objective(s)

In a prospective, observational 12 week, clinical study in adolescent participants who are high consumers of SSB, we will provide sparkling flavored water to participants and ask them to substitute the drinks for SSB. The objective of the study is to demonstrate whether substituting sparkling water flavored drinks for SSB has glycemic and cardiometabolic benefits while maintaining acceptability to adolescents who are at risk for diabetes.

2.1 Primary Objective

Determine the effect of reducing added sugars intake by substituting carbonated sparkling water on T2D risk in adolescents.

Hypothesis: Adolescents who decrease consumption of SSB by substituting sparkling water will experience decreased blood glucose concentrations and increased insulin sensitivity during an oral glucose tolerance test and decreased glucose excursions during continued glucose monitoring, compared to those who do not decrease consumption of SSB.

2.2 Secondary Objective

Determine the acceptability of sparkling water with no added sugars in adolescents.

Hypothesis: *After consuming sparkling water flavored drinks for 12 weeks, adolescents' acceptance of the flavor profile of the drinks will improve.*

2.3 Description of the Intervention to Reduce Intake of SSB by substituting carbonated flavored seltzer water: To encourage participants to substitute sparkling water flavored water for SSB intake, we will provide enough supplies of the drinks to adolescents (and their families) each month. We will also provide iPhones with the TADA app to the adolescent participants. Study staff will remind participants to consume the sparkling water through iMessages sent through the TADA app.

3.0 Outcome Measures/Endpoints

3.1 Primary Outcome Measures

3.1.a Dietary Consumption of SSB and Sparkling Water: We will monitor the adolescents' overall dietary intake using TADA, every two weeks. To assess dietary intake using 4-day food records, we will use the Technology Assisted Dietary Assessment (TADA) system, a mobile app, to acquire food images of eating occasions. TADA was developed at Purdue University and is well suited for the adolescent lifestyle since teens enjoy using mobile technology [25]. We may also assess intake of added sugars by blood biomarkers such as (e.g., $^{13}C/^{12}C$ ratio).

3.1.b 2 hour Glucose Concentrations Measured Using 2 hour Oral Glucose Tolerance Testing: Oral glucose tolerance testing will be performed at the IU or Purdue Clinical Research Centers, in the morning after participants have undergone an overnight fast for 8 hours. Blood samples will be obtained at -15, 0, 15, 30, 60, 90,120 minutes, relative to ingestion of a glucose drink at a dose of 1.75 g/kg body weight (maximum 75 g glucose). Other primary outcomes will be concentrations of fasting glucose and HbA1c concentrations.

3.1.c. Insulin Sensitivity: For measures of insulin action, which takes into account serum glucose and insulin concentrations, serum fractions from the oral glucose tolerance test will be frozen at -80°C until analysis. Glucose concentrations will be determined using an automated chemistry analyzer (COBAS Integra 800, Roche Diagnostics, Indianapolis, IN); insulin, and c-peptide will be assessed using an Elecsys Systems immunoassay analyzer (Roche Diagnostics). Whole-body insulin sensitivity index (WBISI), the insulinogenic index (IGI), and the oral disposition index (DI) will be calculated as described [26, 27]. The homeostasis model assessment of insulin resistance (HOMA-IR) will be calculated from glucose and insulin concentrations in the fasting state [28]. We will also calculate the HOMA%B and %S values using the HOMA calculator https://www.dtu.ox.ac.uk/homacalculator/, which takes into account the variations in hepatic and peripheral glucose.

3.1.d. Glycemic Control Measured Using Continuous Glucose Monitoring: We will collect free-living, glucose measures for 6 consecutive days using the Continuous Glucose Monitoring

(CGM; FreeStyle Libre Pro System; Abbott; Abbott Park, IL[29]. The CGM will be administered to participants 6 days before the start of Week 0 and Week 12, and the monitor will be removed at the clinic visit. For the placement of the CGM, the back of the participant's upper arm will be cleaned with an alcohol wipe and dried. The sensor will be firmly adhered to the arm with the needle application inserted. The FreeStyle sensor measures glucose every 15 sec and records an average glucose value every 15 min for up to 14 days.

3.2 Secondary Outcome Measures

3.2.a. Flavor Perception of Sparkling Water. We will use sensory testing to measure perception of sparkling beverages at the baseline and at the end of the study [30]. We will test a total of 6 levels of sweetness in the beverages so that we can observe if a shift in ideal level of sweetness occurs after consuming the unsweetened waters. The sparkling beverages will be labeled with randomized 3-digit codes. The participants will first taste an unsweetened and fully sweetened beverages (to control for order and desensitization effects) and then all samples (including replicates of the unsweetened and fully sweetened beverages) will be presented to participants in counterbalanced order. Participants will rate the samples for their sweetness using a "just about right" visual analog scale, with anchors at: -100, "Not sweet enough"; -50, "Slightly not sweet enough"; 0, "Just about right"; +50, "Slightly too sweet"; and +100 "Too sweet." Samples will also be rated for liking using a hedonic visual analog scale, with anchors at: -100, "Worst ever"; -50, "Dislike"; 0, "Neutral"; +50, "Like"; +100, "Best ever." Before and between each sample participants will rinse with water, and a 1 minute break will be enforced between each sample.

3.2.b. CVD Outcomes, Blood Triglycerides, Blood Pressure and Waist Circumference. We will assess these measures during study visits at the clinical centers at the same time and under fasting conditions as for the oral glucose tolerance testing. We will measure blood pressure after 5 minutes of rest, on the right arm in the supine position, using a sphygmomanometer with an appropriate sized cuff [31]. Plasma triglyceride concentrations (along with total, and low and high-density cholesterol) will be measured by the Indiana University School of Medicine Diabetes Center Translation Core laboratory (Indianapolis, IN), using established enzymatic colormetric methods. Anthropometric measurements will include height, weight, body mass index, waist circumference, and sagittal abdominal diameter [32]. We will calculate BMI percentiles and Z-scores from age- and sex-specific reference values as defined by the Centers for Disease Control and Prevention (CDC) growth charts [33]..

3.3 Tertiary/Exploratory/Correlative Outcome Measures

3.3.a. Other Dietary Intake Behaviors, as well as Physical Activity and Sedentary Behaviors: Changes in dietary intake of fiber, potassium, magnesium, calcium and saturated fat, as well as in physical activity or interactions between these components may play a role in the observed effects. To create 4-day food records, we will use the TADA mobile app (34). Using linear modeling, we will assess the contribution of these factors as covariates.

4 Eligibility Criteria

4.1 Inclusion Criteria

- Male and female adolescents (age 10-21 years)
- Overweight and Obese (body mass index ≥ 85 percentile for age and sex)
- High consumers of SSB, defined as >2 or more servings per day
- Family history of diabetes in a first or second degree relative OR prediabetes (i.e., evidence of either impaired glucose tolerance (HbA1c 5.7 – 6.4%, or plasma glucose between 140-199 mg/dL at 2 hours on oral glucose tolerance testing) or impaired fasting plasma glucose (≥ 100 mg/dL)) [37].

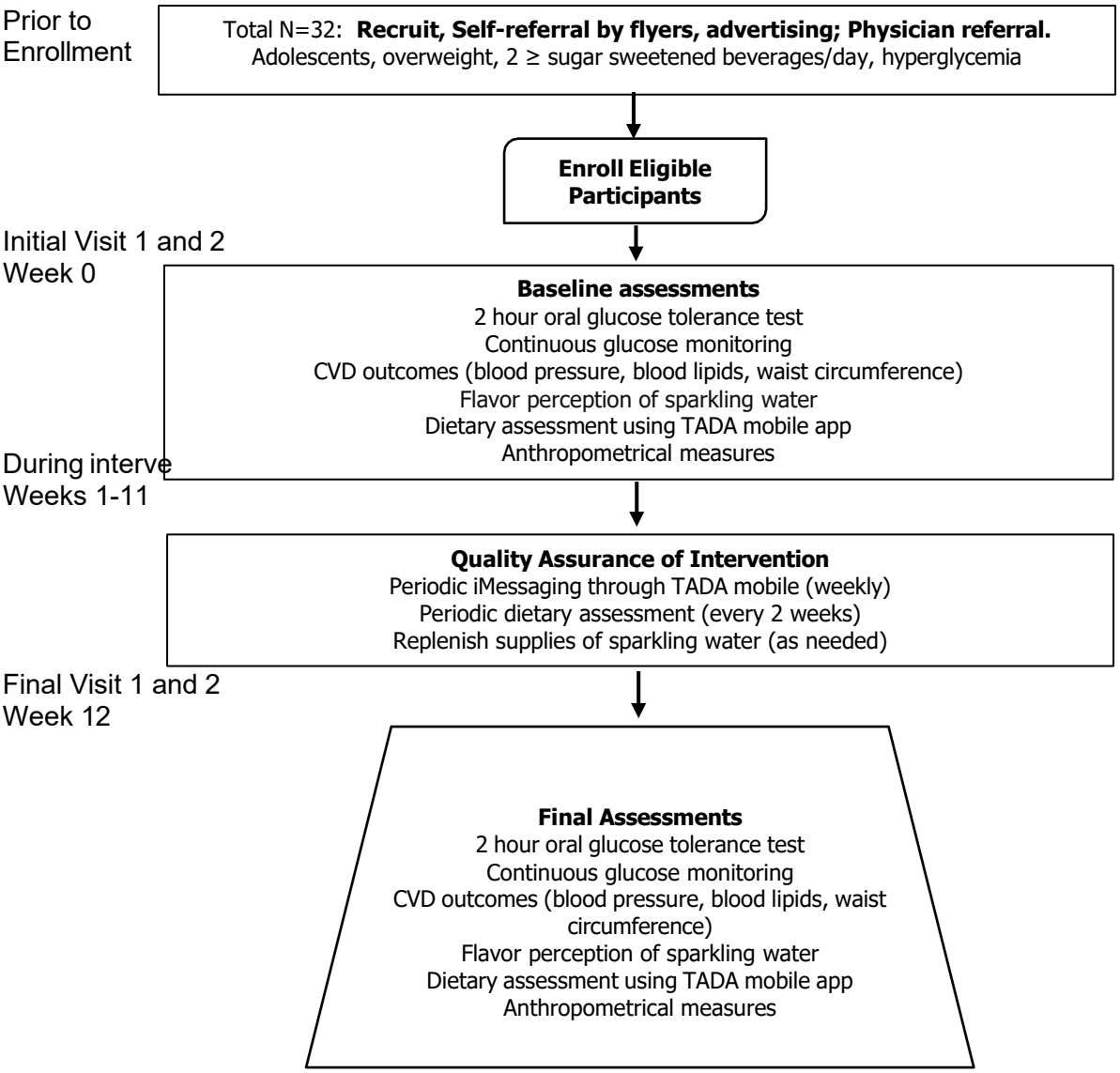
4.2 Exclusion Criteria

- Pregnancy
- Use of medications that affect glucose metabolism (such as glucocorticoid-containing medications or atypical antipsychotics). We will not exclude female participants who currently use, are planning to use, or planning to stop taking oral contraceptives.
- Syndromic obesity (such as Prader Willi, hypothalamic obesity, or Laurence-Moon-Biedl)

5.0 Study Design

The study design is a prospective observational study in adolescents who are at risk for developing T2D, to evaluate the effectiveness of sparkling water to reduce dietary intake of added sugars and thereby improve glycemia. Study data on consumption and the flavor profile of sparkling water will serve as a measure of acceptability. The study intervention will be to provide carbonated flavored sparkling water for 12 weeks to adolescents who have a usual intake of 2 or more servings of SSB per day and are at a high risk for developing type 2 diabetes. Study measures will be obtained before and after the exposure to carbonated flavored sparkling water and each participant will serve as his/her own control. To encourage the participants to substitute the carbonated flavored sparkling water, study personnel will send them weekly iMessages through the TADA app. In addition, we will monitor the participants' diet, using the TADA app, every two weeks, for 4 days. Study measurements will be obtained at baseline, before the intervention, during and at the end of the 12-week intervention.

Flow diagram for randomized, controlled trial



6.0 Enrollment/Randomization

Recruitment: We will recruit participants from the Youth Diabetes Prevention Clinic (Dr. Hannon is the director) and from the community. We will also contact participants who have completed our ongoing DIG IT study and who consented that we could contact them for future studies (IU – IRB Protocol #1403986016). Clinic patients less than 18 years of age may be accompanied by parental guardians who report on the child's demographic information. A dietitian routinely interviews patients about dietary habits, including consumption of sugar sweetened beverages. The information regarding dietary intake of SSB may also be obtained from the Bright Futures Adolescent Supplemental Questionnaire, that we used in previous research [34]. In our experience, 56% of participants responded "yes" to drinking more than one SSB each day and 25% of patients reported that they consumed these beverages 3 or more times per day (35) [34]. We will advertise the study by placing flyers in physician clinics, newsletters and in public spaces (e.g., libraries). We will use a self-referral approach so that those who are interested will contact

the researchers. An informational meeting with the adolescents and their family member will be arranged where the study, procedures, and informed consent will be thoroughly described. After informed consent has been obtained, each volunteer will be asked to complete the screening questionnaire and a clinical profile will be obtained to check for general health. This recruiting plan has been used successfully for recruiting subjects in our ongoing DIG IT study (IU – IRB Protocol #1403986016).

We may use a e-newsletter at Indiana University, Purdue University, or Indiana University-Purdue University Indianapolis (IUPUI) for recruitment. The language for the newsletter is provided in the protocol submission. We may also use a study flyer, posted in public places such as libraries for recruitment. The language for the study flyer is provided in the protocol submission. We may recruit using public advertisements, the language for this included in the protocol submission.

Enrollment: We will enroll 16 participants. In order to enroll the 16 subjects, we will require 32 participants to undergo screening. Participants who are interested will sign informed consent and assent (assent subjects < 14 years of age). A study investigator will review with the participants all risks and benefits from participation in the study.

Randomization: This is a single arm protocol without randomization of study participants. All participants will be provided the sparkling water. Each participant will serve as his/her own control.

7.0 Study Procedures

1. Description of Intervention - Exposure to Sparkling Water: During baseline study visit 1, participants will be provided with 4 flavors of sparkling water (1 of each flavor) to sample in the 6 days prior to the beginning of the study. Each participant will be asked to choose the his/her most preferred flavor, which will be used during the 12-week intervention. The intervention will be to provide sparkling water to all participants and ask them to substitute the water in place of sugar sweetened beverages for 12 weeks. We will work with the parents/guardians to determine whether delivery or pickup of the beverages from a store is most feasible. To encourage and remind the participants to drink the sparkling water, study personnel will send iMessages through the TADA app, once a week. To assess the consumption of the sparkling drinks, as well as total dietary intake, the participants will create food records, using the TADA app, at baseline, every two weeks and at the end of the study.

2. Baseline. Initial Visit One - Obtaining Consent and Glycemia and Dietary Measurements: This meeting will occur at the Clinical Research Center at Indiana University-Purdue University Indianapolis (CRC IUPUI) . The research team will meet with potential subjects to explain the study and to consent/assent the subject. If consent/assent is obtained, the study physician or study nurse will place the continuing glucose monitor. The researcher will give instruction on tracking dietary intake, using the TADA tool. The consent documents may be mailed to the potential participant prior to the training meeting in order to have time to review them. The visit will take one hour of time. During this visit, participants will also be asked about any food allergies and dietary restrictions. This information will be used to select a snack (granola or breakfast bar type item) for participants to consume before the sensory test during baseline visit two and at the final study visit two. Consumption of the snack ensure the participant is not excessively hungry

before making the sensory ratings (hunger makes energy containing foods more palatable, skewing sensory ratings).

3. Placement of the Continuous Glucose Monitor: Free-living, glucose measures will be performed for 6 consecutive days using the Continuous Glucose Monitoring (CGM; FreeStyle Libre Pro System; Abbott; Abbott Park, IL [29]. The CGM will be obtained for 6 days approximately 1 week before the baseline study visit. For insertion of the CGM, a small area on the participant's arm will be cleaned and the tiny glucose sensor will be inserted just under the skin and held in place with Tegaderm tape. The sensor measures glucose every 15 sec and records an average glucose value every 15 min for up to 14 days. The instrument will be placed by a registered nurse or research assistant who has been trained by the manufacturer. The instrument will be used as indicated by the manufacturer and instructions for user safety will be followed. https://www.freestylelibre.us/?source=provider.myfreestyle.com

4. Instructions for the TADA system: Dietary intake will be measured for 4 days using the TADA tool. This device allows individuals to capture food images on mobile devices. Each food item is segmented, identified, and its volume is estimated by a trained professional. The energy and nutrients consumed can be determined by capturing "before" and "after" eating occasion images. Subjects will be provided with an iPhone for purposes of completing daily diaries and recording daily food intake for four consecutive days. Subjects will receive training on use of this tool as a research device, including contact information for research staff should problems with the iPhone rise. Each subject will be instructed and shown how to use the iPhone to take images and to send the images to the data center using software installed on the device. The procedure for taking images of each eating occasion involves the positioning of a marker next to foods and/or beverages to be consumed. When the subject is in a WiFi hotspot, the images are automatically sent to a secure server. The procedure for taking and sending each image is expected to take 10-15 seconds. The TADA system will provide a separate recording process for beverage consumption, where participants will be able to inform what beverage and how much they consumed. The iPhone Location Services option in the settings menu will be turned to "On." This will allow researchers to collect location-based information for increased accuracy during diet data analysis. This feature will enable investigators to identify the recipe components of foods especially when they are consumed in restaurant setting, since the corresponding restaurant will be identified by its location. All information obtained regarding location will only be used by investigators for assessing dietary behavior and will be stored in secured databases. The risks involved in these procedures are no more than what a person would encounter in everyday living. The iPhones will be provided in protective cases so that it is unlikely that they will be damaged if dropped during everyday use. Subjects will not incur any costs associated with the use of the iPhone. Subjects are encouraged to return the iPhone during the subsequent clinic visit with an incentive of a total of $15. The subject should begin using the iPhone for recording daily food intake right away after the consent/assent/training meeting.

5. Baseline Study Visit Two: About 1 week after the CGM and diet measures are obtained, the subject will attend a clinic visit at the CRC IUPUI which is part of the Indiana Clinical and Translational Science Institute. The visit will take 3 1/2 hours of time.

6. Oral glucose tolerance test. Oral glucose tolerance testing will be performed during week 0 and week 12, at the CRC IUPUI in the morning after participants have undergone an overnight fast for 8 hours. Blood samples will be obtained at -15, 0, 15, 30, 60, 90,120

minutes, relative to ingestion of a glucose drink at a dose of 1.75 g/kg body weight (maximum 75 g glucose). The procedure will be performed by trained nurses at the Indiana Clinical and Translational Science Institute. Glucose readings are performed at each timepoint using point of care instruments.

7. Cardiometabolic outcomes: Blood samples for glucose, insulin, and blood lipid concentrations will be obtained in the morning after participants have undergone an 8 hour fast. Anthropometrical measures of obesity including height and weight, the body mass index, waist circumference, and sagittal abdominal diameter, will be obtained on weeks 0 and 12 [32]. Blood pressure will be measured after 5 minutes of rest, on the right arm in the supine position, using a sphygmomanometer with an appropriate sized cuff [31].

8. Sensory testing to measure perception of sparkling beverages. After the other measurements are complete, but before the sensory tests, participants will be provided a snack (granola or breakfast bar) in order to alleviate hunger. Sensory tests for will be administered at the baseline and at the end of the study. The sparkling beverages, with and without sugar, will be labeled with randomized 3-digit codes, and presented to participants in counterbalanced order.
   Participants will rate the samples for their sweetness using a "just about right" visual analog scale, with anchors at: -100, "Not sweet enough"; -50, "Slightly not sweet enough"; 0, "Just about right"; +50, "Slightly too sweet"; and +100 "Too sweet." Samples will also be rated for liking using a hedonic visual analog scale, with anchors at: -100, "Worst ever"; -50, "Dislike"; 0, "Neutral"; +50, "Like"; +100, "Best ever." Before and between each sample participants will rinse with water, and a 1-minute break will be enforced between each sample.

9. Final Study Clinic Visit One: At the end of week 11 and start of week 12, the participant and family member will attend a visit at the CRC IUPUI where the study team will obtain CGM and TADA measures. This visit will be a repeat of Baseline Study Visit 1. The visit will take 1 hour of time.

10. Final Study Clinic Visit Two: At the end of the 12 week study, we will assess the study outcomes during a clinic visit, identical to the baseline study visit 2. We will perform oral glucose tolerance testing, cardiometabolic outcomes, and flavor perception of the sparkling waters. The visit will take 3 1/2 hours of time.

8.0 Study Calendar

STUDY PROCEDURES	**Baseline Study Visit One**	**Baseline Study Visit Two**	**Follow-up**					**Final Study Visit One**	**Final Study Visit Two**
	-2 wk	Day 0	2 wk	4 wk	6 wk	8 wk	10 wk	11 wk	Week 12
Consent/Assent	X								
CGM monitoring	X							X	
TADA Dietary Assessment	X		X	X	X	X	X	X	
Oral Glucose Tolerance Testing		X							X

Cardiometabolic outcomes		X							X
Sensory Testing		X							X

To allow for deviations in terms of timing and scheduling of study visits, we will consider variations of +/- 7 days from the scheduled visit.

9.0 Reportable Events

We will report adverse event or unanticipated problems involving risk to participants or other to the IU Human Subjects Office promptly and according to the guidance provided by the IU HRPP Reportable Events Policy and Guidance. Adverse events will be reported promptly, within 5 business days of the study team becoming aware of the event, if they are assessed by the PI as (1) unexpected, (2) related or possibly related to study participation, AND (3) suggests that the research places subject(s) or others at greater risk of harm than was previously known. Collection of adverse events (AE) and serious adverse events (SAE) will begin at the start of interventions.

For this study, the following standard AE definitions are used:

Adverse Event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure, regardless of whether it is considered related to the medical treatment or procedure.

Serious Adverse Event: Any AE that results in any of the following outcomes:

- Death
- Life-threatening
- Event requiring inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant disability/incapacity

AEs are graded according to the following scale:

Mild: An experience that is transient and requires no special treatment or intervention. The experience does not generally interfere with usual daily activities. This includes transient laboratory test alterations.

Moderate: An experience that is alleviated with simple therapeutic treatments. The experience impacts usual daily activities. Includes laboratory test alterations indicating injury, but without long-term risk.

Severe: An experience that requires therapeutic intervention. The experience interrupts usual daily activities. If hospitalization (or prolongation of hospitalization) is required for treatment it becomes an SAE.

The study uses the following AE attribution scale:

Not related: The AE is clearly not related to the study procedures (i.e., another cause of the event is most plausible and/or a clinically plausible temporal sequence is inconsistent with the onset of the event).

Possibly related: An event that follows a reasonable temporal sequence from initiation of study procedures, but could readily have been produced by a number of other factors.

Related: The AE is clearly related to the study procedures.

AEs are identified during study visits when subjects are interviewed about recent medical history and a physical examination of the subject. After discharge, AEs are assessed at time of study follow-up visits.

SAEs and specific procedure-associated AEs are reported to the IRB within 24 hours. In addition, all AEs are reported according to the IRB using AE reporting guidelines

Management of risks to the subjects

Expected AEs – Related to Consumption of Sparkling Water

Expected AEs associated with drinking sparkling include: abdominal pain (with acute ingestion of large volumes) or increased tooth sensitivity (studies have shown that sparkling waters minimally erode the enamel of teeth (36)). We expect that occurrence of these adverse event to be rare. According to the American Dental Association, sparkling water has a similar effect on teeth as regular non-carbonated water.

AE Management

We expect study participants to consume sparkling water at levels like consumption of carbonated sodas (e.g. more than 2 drinks per day). We will counsel participants to consume the sparkling water at the rate typical for their consumption of sodas, and to not over consume the sparkling water. If participants experience abdominal pain or tooth sensitivity, we will ask them to discontinue their consumption.

Expected AEs – Related to Blood Draws

Expected AEs associated with blood draws are pain, bruising and lightheadedness. Rarely infection and fainting can occur with drawing blood.

AE Management

Research nurses at the Clinical Research Center and similarly trained study personnel who are experienced in phlebotomy will conduct blood draws.

Expected AEs – Related to oral glucose tolerance testing

Expected AEs associated with OGTT the feeling of nausea after drinking the glucose drink. Pain and small risk of skin bruising, bleeding and infections associated with placement of the catheter and blood draws. Rarely infection, lightheadedness and fainting can occur with drawing blood.

AE Management

Research nurses at the Clinical Research Center and similarly trained study personnel who are experienced in phlebotomy will conduct blood draws.

Expected AEs – Related to continuous glucose monitoring

Expected AEs associated with CGM are a small risk of infection, bruising or nausea during placement of the monitor.

AE Management

To minimize risk, the placement of the CGM will be performed with sterile techniques by the study physician or study nurse.

Expected AEs – Related to monitoring of dietary intake

Expected AEs associated with using instruments to collect dietary intake (TADA, 4 day food records) are feelings of discomfort related to privacy and confidentiality. These tests have been used in previous research, and no adverse events were noted (34).

AE Management
The data is subject to the rules under the Health Insurance and Portability and Accountability Act (HIPPA) regulations (see **Confidentiality** below).

Expected AEs – Related to sensory testing
Expected AEs associated with sensory testing should not exceed those of everyday life, especially for those who drink carbonated beverages. The tests will involve consuming a snack and then tasting small volumes (30 mL) of carbonated beverages with various concentrations of sugar within ranges that are commercially available.

AE Management
We will determine food intolerances or allergies to any ingredient in the drinks before administering the drinks.

10.0 Data Safety Monitoring

Adverse events will be monitored by the co-PIs via notification of the principal investigator by the research subject and by regular review of safety and research labs (at each study visit). Each event will be handled individually. Adverse events or unanticipated problems involving risk to the subjects or others will be reported according to IRB standard operating procedures. If the adverse event represents an unanticipated problem and requires changes to the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others, it will be reported to the IRB using the Prompt Reporting Form within five business days of the investigator becoming aware of the event. Unless determined to not represent an unanticipated problem by RCA staff or the IRB Chair or Chair's designee, the report will be reviewed at a convened IRB meeting for possible action. Any serious unanticipated adverse events will be reported within 24 hours of becoming aware of the event. Any new diagnosis found during a study visit, such as type 2 diabetes, hypertension, or high cholesterol, will be reported to the study subject by Dr. Tamara Hannon, MD or trained research staff member, and appropriate referral will be made for treatment.

Potential Risks:
The risks of the study protocol as outlined are considered minimal to the participants.

Consumption of sparkling waters: We expect minimal risks regarding consumption of sparkling waters. For adolescents, who are high consumers of sugar sweetened sodas, the risks of consumption of sparkling waters are no more than those for everyday living.

Blood draws: No more than 150 mL total per subject per study or <3 mL/kg body weight, will be collected, which is the amounts allowed by NIH for children. Blood draws will occur after an 8 hour overnight fast and are likely to be via an antecubital vein and will be used to assess glucose, insulin, blood lipid concentrations and biomarkers of added sugar intake (e.g., $^{13}C/^{12}C$ ratio). Pain and bruising and much less likely infection could occur with venipuncture. Lightheadedness and rarely, fainting can also occur with drawing blood.

2 hour oral glucose tolerance testing. The risks associated with an OGTT relate to the placement of a catheter, frequent blood draws over a two hour period and drinking concentrated glucose (75g per serving). This test is frequently done in the clinical and research settings, including in the adolescent population by our research group. The risks to subjects are anticipated to be minimal. There is a small risk of skin bruising, bleeding and infections associated with blood draws from a catheter. There is a small risk of feeling nausea after drinking the glucose drink.

Glucose readings are performed at each timepoint using point of care instruments to monitor for the possibility of hyper and hypoglycemia.

Assessment of Cardiovascular Outcomes: Blood pressure and Waist Circumference: Each of these measures are conducted frequently in the clinical setting and are considered to be minimal in risk to participants.

Continuous glucose monitoring: The continuous glucose monitoring system (CGM) is worn to measure how blood sugar levels change over the day in a free-living environment. A video demonstration is provided here: https://www.freestylelibre.us/?source=provider.myfreestyle.com The CGM is worn on the back of upper arm. It is a tiny glucose sensor will be adhered to the arm with a needle inserted just under the skin. We will clean the area and insert the instrument by clamping it under the skin. Tape on the instrument will hold it in place when the participant is wearing it. This device measures and records blood glucose values throughout the day. There is a small risk of infection, bruising or nausea associated when using the continuous glucose monitoring system, however, it does not interfere with regular daily activities. To minimize risk, the placement of the CGM is performed with sterile techniques by the study physician or study nurse.

Monitoring of dietary intake: Subjects will use instruments designed to collect dietary intake (TADA, 4 day food records). The risks involved in these tests do not exceed those of normal, everyday life, especially for those who use cell phones and interact frequently with wearable technology. These tests have been used in previous research, and no adverse events were noted [35]. Risks associated with these procedures are related to confidentiality and privacy. Answering questions may cause feelings of anxiety and discomfort. The data is subject to the rules under the Health Insurance and Portability and Accountability Act (HIPPA) regulations (see **Confidentiality** below).

Sensory Testing: The tests will involve consuming a snack and then tasting small volumes (30 ml) of carbonated beverages with various concentrations of sugar within ranges that are commercially available. For this reason, we expect this testing to involve minimal risk to the participants. We will exclude participants from the study who are allergic or intolerant to the items that we are testing.

Participant Safety: Adolescents will always be accompanied by a guardian for all study visit at the Clinical Research Centers. All clinical protocols are conducted in the Clinical Research Centers under the supervision of a study physician or a research nurse. All members of the study team will undergo CITI training for Biomedical Researchers as well as Good Clinical Practice.

Confidentiality: Participants and their guardian will be asked demographic, family history and other questions related to their health. The loss of confidentiality is related to normal risk of providing similar information in a clinic setting.

A. Adequacy of Protection Against Risks

Protection Against Risks:

Experimental procedures and consent forms will be done in accordance with the Institutional Review Boards of Indiana University and Purdue University. The experimental procedure is described on the consent form and is to be signed before the start of any procedure. The signed consent forms will be stored in our permanent record files. Participants are clearly informed that

they may stop at any time and that they may contact a study investigator directly if there are any adverse effects or concerns.

The study physician (Dr. Hannon) will administer an appropriate physical exam during screening to ensure all volunteers are relatively healthy. If a child has markedly abnormal laboratories or other measures during screening, he/she will be referred to a physician (the child's PCP and/or TH) immediately for evaluation and treatment. Continuation in the study will be at the discretion of their physician and the clinical and safety team.

Inclusion and Exclusion criteria: Our strict inclusion and exclusion criteria for entry will minimize risks.

These criteria will be evaluated during a screening visit and are listed below:

The inclusion criteria: 1) Girls and boys of all race/ethnic groups, aged 10 to 21 years, who are overweight and obese and have a family history of diabetes or have prediabetes; as defined by the American Diabetes Association [37] 2) Have signed informed consent/assent.

The exclusion criteria are: 1) participant is diagnosed with normal glucose tolerance (NGT); 2) participant is diagnosed with T2D; 3) participant is pregnant; 4) use of medications that affect glucose metabolism (such as glucocorticoid-containing medication or atypical antipsychotics). We will not exclude female participants who are currently using, planning to use, or planning to stop using oral contraceptives; 5) syndromic obesity (such as Prader Willi, hypothalamic obesity, or Laurence-Moon-Biedl).

Training of study personnel: A study nurse will be trained to place the continuous glucose monitor. Trained nurses or phlebotomists at the I-CTSI will perform catheter placement and/or blood draws. Clinical procedures within the I-CTSI facilities will be overseen by a study physician. All research personnel will undergo human subjects CITI training.

Additional Protections Against Risk: Participants may refuse to answer questions on questionnaires or on phone calls with the study personnel. During the OGTT, glucose readings will be performed at each timepoint using point of care instruments to monitor for the possibility of hyper and hypoglycemia. Participants will be asked about any food intolerances or allergies to ingredients before snacks are provided prior to the sensory tests. A snack that offers no risk to participants will be chosen. Adolescents will always be accompanied by a guardian for all study visit at the Clinical Research Center. Regarding using the TADA app on an iPhone, subjects will not incur any costs associated with the use of the iPhone. The iPhones will be provided in protective cases so that it is unlikely that they will be damaged if dropped during everyday use.

11.0 Study Withdrawal/Discontinuation

Subjects will be withdrawn from the study for failure to comply with study procedures of if they request to withdraw. Criterial for withdrawal include failure to comply with the study procedures, pregnancy, initiation of medications that affect glucose metabolism (such as atypical antipsychotics or steroids), or development of a disease that affects glucose metabolism, such as a chronic disease. In these instances, referral to appropriate specialty care will be made.

12.0 Statistical Considerations

Power analysis and Estimation of Sample Size: The sample size required for the study is based on the power calculation for the primary outcomes of 2 hour glucose concentrations. Based on preliminary our data from our ongoing DIG IT Study, IU-IRB 1403986016, we show that by reducing added sugars from 9.0% to 6.6% of energy leads to a decrease in 2 hour glycemia of 143.9 ± 9.6 to 123.7 ± 22.6 mg/dL, which is statistically significant and clinically meaningful. Specifically, a sample size of 13 will enable us to detect differences in 2 hr glucose concentrations before and after the intervention, with 80% power and $\alpha = 0.05$. With an expectation of 20% drop out, we will enroll 16 participants.

We will test the data for normalcy and calculate descriptive statistics. Skewed data will be normalized or non-parametric statistics will be used. For Aim 1, changes in glycemia, peak glucose excursions and insulin sensitivity will be assessed using paired T-tests (for normally distributed data). For Aim 2, changes in intensity of sweetness and liking for sparkling water will be assessed using mixed linear models with the participant as a repeated factor. As a secondary analysis we will assess relationship between changes in perceptions of sparkling water, SSB consumption and glycemic measures.

13.0 Statistical Data Management

Primary data will be collected via paper, phone interviews, direct electronic data capture from the TADA mobile app and stored electronically in REDCap, secure IT servers within IU-Bloomington. The storage location will be backed up automatically every day. Quality assurance steps will include: 1) built in range checks; and 2) testing of database by study team prior to moving to production mode. The following quality control methods will be used: 1) single entry with random checks of accuracy]; and 2) extraction and cleaning of data that will be used for analysis every 1 year.

14.0 Privacy/Confidentiality Issues

All paper records containing identifying information will be kept in locked offices. All computer records utilized for the study will require password access. Information utilized for study data may be stored in one or more of the following: excel spreadsheets, statistical software databases, RedCap, PResNet database. Access to the study data will be restricted to study personnel.

15.0 Follow-up and Record Retention

The duration of study record retention will be at least 7 years after the study has ended, with the possibility of indefinite archiving of study records and data.

16.0 COVID-19 Contingency Plan

Given the current situation of coronavirus and social distancing recommendations, we would also like to propose the following alternative option for our research approach, utilizing remote execution of procedures that can be performed virtually.

16.1 Remote Enrollment

We will continue the original plan of recruitment, from the Youth Diabetes Prevention Clinic, the community, as well as participants that have completed the ongoing DIG IT study and who consented that we could contact them for future studies (IU – IRB Protocol #1403986016). In

addition to these methods of recruitment, we will use social media advertisements (Facebook and Instagram) and online flyers of the study as additional sources for remote recruitment.

16.2 Study Procedures

The study procedures would be conducted in three different online sessions. The purpose of the online sessions is to ensure participants and their parents/guardians have full understanding of the study and perform the tasks as instructed, minimizing any potential risks and maximizing accuracy of results. Each online session would have up to 1 hour in duration. Details for each session are noted below.

Session 1

1. **Consent/Assent forms.** Consent/assent forms will be mailed to potential participants. During the online session, a study investigator will review with participants and their parent/guardians all the risks and benefits of participating in the study. Upon decision of participating in the study, participants older than 14 years of age will sign the consent forms, and participants younger than 14 years will have their parents/guardians sign the assent forms. If participants consent/assent, they will sign the forms, send immediate pictures of the signature pages, and then return to us physically using a provided stamped envelope for return. If a participant does not consent/assent, we will end the session.

2. **TADA instructions.** The TADA app will be previously installed on the iPhones provided to participants. After consent/assent, participants will be instructed on how to use the app to report their consumption of flavored seltzer water and other beverages, as well as how to take pictures of their food for the 4-day food record (every 2 weeks).

3. **Anthropometric measurements.** Participants will be asked to weigh themselves and measure their height with the help of another person. They will also be guided on how to measure their waist circumference, as previously described (38). If participants do not have a scale or measuring tape, these items will be delivered to participants' houses.

4. **Choosing seltzer water and soda flavors for sensory tests and 12-week intervention.** Participants will be given the option of choosing flavored sparkling water. Sugar-sweetened soda of the matching flavor will be used in the sensory tests.

In the second session, participants and their parents/guardians will be instructed on how to perform the sensory tests. After instructions are provided, the participants will execute the sensory tests, while the study personnel remains online to witness the test and provide any assistance needed by participants. Session 2 is described in further details below. Session 3 will be equal to session 2 and will happen at the end of the 12-week intervention.

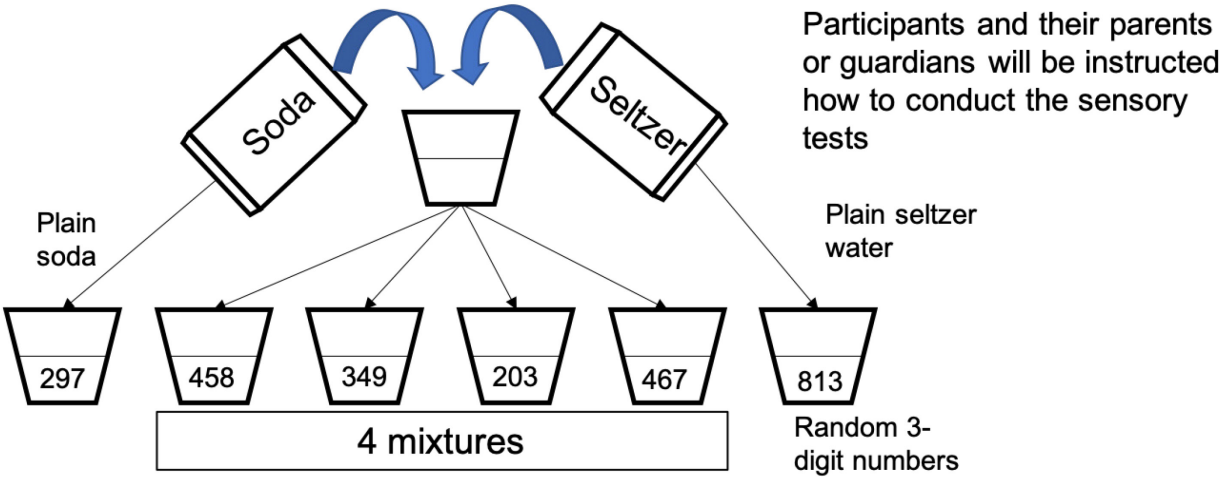
Session 2

Sensory testing to measure perception of sparkling beverages. Due to the current situation with the COVID-19 pandemic, we intend to execute the sensory testing remotely. An online platform, such as Zoom, Cisco Webex, Skype or other will be used to instruct the participants and a parent or guardian about how to perform the sensory tests. No video recordings or pictures will be taken of the participants. We will ask the participants to conduct the test when they are not hungry, but when they have not consumed another food or beverages (other than water) for at least 1 hour. . Flavored sparkling water and sodas will be used to conduct the sensory tests. The beverages will be ordered online

from supermarkets such Walmart, Target or Payless and the participants will have the options of home delivery or store pickup. A marked cups will also be delivered so that the parents or guardians of participants can mix the correct volume of sparkling water and soda to obtain a total of 6 levels of sweetness (plain flavored sparkling water, plain soda and 4 mixtures of sparkling water and soda that vary in sweetness intensity) in the beverages. Disposable plastic cups will also be delivered to participants to test the 6 sweetness levels. We will guide the parents through the testing procedure, instructing them which sample to give when (in order to maintain randomization of sample tasting). Participants will rate the samples for their sweetness using a "just about right" visual analog scale, with anchors at: -100, "Not sweet enough"; -50, "Slightly not sweet enough"; 0, "Just about right"; +50, "Slightly too sweet"; and +100 "Too sweet." Samples will also be rated for liking using a hedonic visual analog scale, with anchors at: -100, "Worst ever"; -50, "Dislike"; 0, "Neutral"; +50, "Like"; +100, "Best ever." Before and between each sample participants will rinse with water, and a 1-minute break will be enforced between each sample. These ratings will be collected digitally using the same iphones that we will use for the TADA app.
Below is a scheme of the remote sensory test.

Session 3

Sensory testing to measure perception of sparkling beverages. Due to the current situation with the COVID-19 pandemic, we intend to execute the sensory testing remotely. An online platform, such as Zoom, Cisco Webex, Skype or other will be used to instruct the participants and a parent or guardian about how to perform the sensory tests. No video recordings or pictures will be taken of the participants. We will ask the participants to conduct the test when they are not hungry, but when they have not consumed another food or beverages (other than water) for at least 1 hour. . Flavored sparkling water and sodas will be used to conduct the sensory tests. The beverages will be ordered online from supermarkets such Walmart, Target or Payless and the participants will have the options of home delivery or store pickup. A marked cups will also be delivered so that the parents or guardians of participants can mix the correct volume of sparkling water and soda to obtain a total of 6 levels of sweetness (plain flavored sparkling water, plain soda and 4 mixtures of sparkling water and soda that vary in sweetness intensity) in the beverages. Disposable plastic cups will also be delivered to participants to test the 6 sweetness levels. We will guide the parents through the testing procedure, instructing them which sample to give when (in order to maintain randomization of sample tasting). Participants will rate the samples for their sweetness using a "just about right" visual analog scale, with anchors at: -100, "Not sweet enough"; -50, "Slightly not sweet enough"; 0, "Just about right"; +50, "Slightly too sweet"; and +100 "Too sweet." Samples will also



be rated for liking using a hedonic visual analog scale, with anchors at: -100, “Worst ever”; -50, “Dislike”; 0, “Neutral”; +50, “Like”; +100, “Best ever.” Before and between each sample participants will rinse with water, and a 1-minute break will be enforced between each sample. These ratings will be collected digitally using the same iphones that we will use for the TADA app.

Anthropometric measurements. Participants will be asked to weigh themselves and measure their height with the help of another person. They will also be guided on how to measure their waist circumference, as previously described (38). If participants do not have a scale or measuring tape, these items will be delivered to participants’ houses.

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