Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Effects of a non-nutritive sweetener restriction intervention in pregnancy and lactation on maternal and infant outcomes (the SweetPea trial): a study protocol for an individually randomized controlled trial

Allison Sylvetsky

allison.sylvetsky@uri.edu

University of Rhode Island

Matthew D. Barberio

Milken Institute School of Public Health, George Washington University

Victoria S. Brownlee

Milken Institute School of Public Health, George Washington University

Emily R. Smith

Milken Institute School of Public Health, George Washington University

Adam Ciarleglio

Milken Institute School of Public Health, George Washington University

Nicholas A. Foreman

Milken Institute School of Public Health, George Washington University

John van den Anker

Children's National Hospital

Jeanne Murphy

George Washington University

Keith A. Crandall

Milken Institute School of Public Health, George Washington University

Ali Rahnavard

Milken Institute School of Public Health, George Washington University

Carmen P. Ortega-Santos

Milken Institute School of Public Health, George Washington University

Molayo Ifebajo

Milken Institute School of Public Health, George Washington University

Nancy Gaba

The George Washington University School of Medicine and Health Sciences

Peter Walter
National Institute of Diabetes and Digestive and Kidney Diseases, NIH
Michael I. Goran
University of Southern California

Study protocol

Keywords: non-nutritive sweeteners (NNS), pregnancy, lactation, infant adiposity, gestational diabetes, glucose tolerance

Posted Date: November 20th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9234409/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Title: Effects of a non-nutritive sweetener restriction intervention in pregnancy and lactation on maternal and infant outcomes (the SweetPea trial): a study protocol for an individually randomized controlled trial

Author Names: Allison Sylvetsky[1,2‡], Matthew D. Barberio[1], Victoria S. Brownlee[1], Emily R. Smith[3], Adam Ciarleglio[6], Nicholas A. Foreman[1], John van den Anker[4], Jeanne Murphy[5], Keith A. Crandall[6], Ali Rahnavard[6], Carmen P. Ortega-Santos[1], Molayo Ifebajo[1], Nancy Gaba[7], Peter Walter[8], Michael I. Goran[9]

‡ Corresponding author

Affiliations:

[1]Department of Exercise and Nutrition Sciences, Milken Institute School of Public Health, George Washington University, Washington, DC, United States

[2]Department of Nutrition, College of Health Sciences, The University of Rhode Island, Kingston, RI, United States

[3]Department of Global Health, Milken Institute School of Public Health, George Washington University, Washington, DC, United States

[4]Center for Health Outcomes Research and Delivery Science, Children's National Hospital, Washington, DC, United States

[5]School of Nursing, George Washington University, Washington, DC, United States

[6]Department of Biostatistics and Bioinformatics and Computational Biology Institute, Milken Institute School of Public Health, George Washington University, Washington, DC, United States

[7]Obstetrics and Gynecology, The George Washington University School of Medicine and Health Sciences, Washington, DC, United States

[8]Clinical Mass Spectrometry Laboratory, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD, United States

[9]Department of Pediatrics, Children's Hospital Los Angeles, University of Southern California, Los Angeles, California, United States

Corresponding Author:

Author: Allison Sylvetsky, PhD

Department of Nutrition

The University of Rhode Island

41 Lower College Road

Suite 130

Kingston, RI 02881

Email: allison.sylvetsky@uri.edu

Phone: 401-874-9324 (office)

Protocol Version: 10 November 2025

Funding: This study is funded by the National Institute of Child Health and Human Development (NICHD). The grant number is: 1R01HD107427-01A1 (PI: Sylvetsky). The sponsor did not have any role in the design or conduct of the trial.

Abstract

Background. Non-nutritive sweeteners (NNS) use is common during pregnancy and lactation, exposing infants *in utero* and through human milk. Evidence from rodent models and human observational studies demonstrates intergenerational NNS exposure may adversely impact metabolic health, increasing the risk for future obesity and cardiometabolic disease.

Methods. We will conduct a parallel, three-arm individually randomized controlled trial with 324 pregnant women who report consuming at least seven 12-ounce servings of beverages with NNS weekly. Participants will be randomized to usual NNS consumption (control), NNS restriction during lactation, or NNS restriction during both pregnancy and lactation. The design is open label, with only outcome assessors blinded; the research team must know the allocation to provide relevant counseling and intervention materials and participants need to be aware of their assigned regimen.

The co-primary outcomes are maternal glucose tolerance, measured by incremental area under the curve (iAUC) during a 75 g oral glucose tolerance test (OGTT) and infant adiposity (fat mass percentage) at 6 months, measured using air displacement plethysmography. Secondary outcomes include: maternal composite whole-body insulin sensitivity index (WBISI) and homeostasis model assessment of insulin resistance (HOMA-IR) at 28 weeks; maternal fasted, 60 min, and 120 min glucose concentrations at 28 weeks; maternal dietary intake at 28 weeks and 1 and 6 months postpartum;

infants' birthweight and gestational age; infants' fat adiposity at 1 month, and infants' weight-for-age and length-for-age at 1 and 6 months. Exploratory outcomes include maternal gestational weight gain during pregnancy, maternal waist circumference at 1 and 6 months postpartum; infants' gut microbiome composition and gut-associated metabolites at 1 and 6 months of age. A complete case, intention-to-treat analysis will be used for the main analysis, and per-protocol analysis removing non-adherent participants will also be performed. We will use a linear mixed effects model with a subject-specific random intercept for multiple observations from the same participant. The Benjamini-Hochberg procedure will be used for multiple comparisons.

Discussion. This is the first randomized controlled trial to investigate the effects of maternal NNS restriction on maternal and infant metabolic health in humans. Findings will inform dietary recommendations regarding NNS consumption during pregnancy and lactation.

Keywords: non-nutritive sweeteners (NNS), pregnancy, lactation, infant adiposity, gestational diabetes, glucose tolerance

Structured Summary

Design: Three-arm, parallel-group randomized controlled trial.

Participants: Pregnant adults meeting prespecified eligibility criteria.

Intervention: Non-nutritive sweetener restriction intervention initiated at 12–18 weeks' gestation; Non-nutritive sweetener restriction intervention initiated at birth

Comparator: Continued usual intake of non-nutritive sweeteners.

Primary outcomes: Maternal glucose tolerance at 28 weeks; infant adiposity at 6 months of age.

Randomization: 1:1:1 allocation.

Sample size: N = 324

Setting: Washington D.C., United States

Trial registration: ClinicalTrials.gov. Identifier: NCT06548828. Date of registration: August 12, 2024.

Background

Non-nutritive sweeteners (NNS), such as aspartame, sucralose, saccharin, stevia, and acesulfame potassium (ace-k) provide sweetness without calories and are commonly used replacements for added sugars in foods and beverages. Over 30% of pregnant women[1] and 44% of lactating women[2] in the United States consume NNS, and infants are exposed to NNS *in utero*[3] and postnatally via breast milk.[4] However, the metabolic and health effects of intergenerational NNS transmission are not well-understood[5] and dietary guidance for or against NNS consumption during pregnancy and lactation is presently lacking.

While the safety of NNS is well-established from a toxicological perspective,[6] accumulating evidence from rodent models[7,8] and human observational studies[9,10] demonstrates that gestational exposure to NNS may adversely impact risk factors for obesity and cardiometabolic disease. Furthermore, maternal NNS consumption during pregnancy is associated with the development of gestational diabetes mellitus (GDM).[11] Aberrant glucose control in pregnancy (i.e., GDM) affects 17% of pregnancies worldwide and GDM is associated with increased infant adiposity and later childhood obesity risk.[12]

Several prospective cohort studies have reported associations between NNS consumption during pregnancy, gestational diabetes, and increased infant and/or childhood BMI.[9,11,13] In rodents, NNS exposure during pregnancy and/or lactation, at physiologically relevant dosages, results in maternal glucose intolerance[8] and gut dysbiosis[7] in the offspring. Aspartame intake during pregnancy has been shown to increase offspring fasting glucose, LDL cholesterol and triglycerides, and impair glucose

tolerance in rodents. Gestational consumption of ace-K has also been shown to increase adult offspring's preference for sweet taste,[14] suggesting that effects could be driven by alterations to central reward systems. Furthermore, compelling evidence, in non-pregnant rodents[15-18] and humans[15,19] demonstrates that NNS exposure disturbs the gut microbiome, which may in part explain reported adverse effects of NNS on obesity and cardiometabolic disease risk factors.[20]

However, all human studies, to date, investigating the relationship between maternal NNS intake and infant outcomes, have been observational and thus prone to reverse causality and residual confounding. Further, most studies have examined weight-based measures such as body mass index (BMI), which may not accurately reflect adiposity, and no human randomized controlled trials (RCTs) have examined effects of NNS consumption on maternal glucose tolerance during pregnancy or infants' gut microbiota or fecal metabolite profiles. This is concerning because the use of NNS in the global food supply is increasing,[21] yet effects of NNS exposure on infant and child health remain poorly understood. Given that early life exposures impact future development,[22] NNS exposure *in utero* and/or postnatally via breast milk may adversely impact developmental programming of cardiometabolic risk.

This RCT ("the SweetPea trial") will therefore examine the effects of restricting habitual NNS consumption during pregnancy and/or lactation on maternal glucose tolerance during pregnancy and infants' adiposity at 6 months of age, along with a variety of other secondary and exploratory maternal and infant outcomes.

Methods

Study design

We will conduct an individually randomized (1:1:1 allocation ratio), single-blinded, parallel group, three-arm trial with pregnant women who report habitual consumption of at least seven 12-ounce servings of NNS-containing beverages per week. Pregnant individuals will be randomized to one of three groups and followed, along with their infant, through 6 months postpartum. Group 1 will not receive any NNS intervention (control; continue usual NNS intake during pregnancy and lactation and receive home safety intervention). Group 2 will continue usual NNS consumption and receive a home safety intervention during pregnancy but will receive an NNS-restriction intervention during lactation. Group 3 will receive an NNS-restriction intervention during both pregnancy and lactation.

This trial protocol was written in accordance with the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) checklist. An overview of the schedule of assessments is shown in **Figure 1** and the full SPIRIT checklist is enclosed in **Supplemental Table 1.**

Study setting

This study will take place at a private university in Washington D.C., which is a city and the U.S. capital. Washington D.C. has a diverse population and over 700,000 residents. In-person study visits will take place in the Exercise and Nutrition Science Laboratories at the George Washington University Milken Institute School of Public

Health in Washington D.C., United States. Screening and monthly consultations will take place virtually and dietary recalls will be conducted by telephone.

Eligibility criteria and recruitment

For recruitment, we will use a combination of in-person and virtual/online approaches, as has been successful in our prior studies.[23,24] Recruitment will take place in waiting rooms of the Ob/Gyn, Maternal Fetal Medicine, and Midwifery clinics at the GW Medical Faculty Associates (MFA). Research staff will be on-site and will share the study information with all patients less than 16 weeks 6 days gestation during their routinely scheduled prenatal appointments. Study information will also be posted in exam and ultrasound rooms, as well as clinic hallways and computer stations. We will also reach the GW MFA patient population virtually through posting study advertisements on *Babyscripts,* a mobile phone application that offers comprehensive maternity care and customizable campaigns for large-scale outreach to a clinic's patient population. In addition, study information will be posted on social media and neighborhood listservs. Study advertisements will contain brief information about the study and a QR code to complete the pre-screening survey for initial eligibility determination. We will also partner with BuildClinical, a professional clinical research recruitment firm to further extend outreach virtually using social media.

The inclusion criteria are as follows: Participants must be pregnant at less than or equal to 16 weeks 6 days gestation (16w6d), have a singleton pregnancy, report habitual consumption of seven or more 12-ounce servings of beverages containing NNS per week, be 18-45 years of age, able to read English at a 5th grade level, and intend to

breastfeed for at least the first 6 months postpartum. For infants, the mother must be enrolled and provide consent for the infant to participate.

The exclusion criteria include: physical or mental concerns preventing study participation; current medication use that may affect body weight, body composition, insulin resistance or lipid profiles; tobacco or drug use during pregnancy; alcohol consumption (>1 drink per week) during pregnancy; history of bariatric surgery; pre-existing gastrointestinal, inflammatory, or malabsorptive disorders such as Crohn's disease, ulcerative colitis, and inflammatory bowel disease that may impact NNS absorption or gut microbiota and known suspected or confirmed genetic fetal abnormalities or suspected or known congenital birth defects.

Research staff will assess eligibility using a two-phase approach. Interested individuals will first complete a brief, electronic pre-screening survey, which will be administered using REDCap[25] and designed to assess potential participants' NNS-containing beverage consumption and initial study eligibility. Prospective participants who are potentially eligible based on their responses to the pre-screening survey will be invited to attend a virtual screening visit with a research staff member. During the virtual screening visit, eligibility will be confirmed, research staff will discuss the study procedures in more detail, and informed consent will be obtained electronically using REDCap[25]. Participants who consent to participate during the virtual screening visit will be scheduled for a baseline study visit, which will take place between 12-18 weeks gestation. Participants will also be asked to provide consent to be contacted regarding participation in future studies. The PRO-EDI table, describing the data collected about participant characteristics, is shown in **Table 2**.

Interventions

Eligible pregnant women will be randomly assigned to one of three treatment groups; Group 1 will not receive any NNS intervention (control; continue usual NNS intake during pregnancy and lactation and receive home safety intervention). Group 2 will continue usual NNS consumption and receive a home safety intervention during pregnancy but will receive an NNS-restriction intervention during lactation. Group 3 will receive an NNS-restriction intervention during both pregnancy and lactation.

Both interventions (NNS restriction and home safety control) will be delivered during four in-person study visits (two during pregnancy, two postpartum), monthly virtual consultations, twice-monthly home deliveries and unsweetened beverages (NNS restriction groups only), and weekly text message reminders.

For NNS restriction, the intervention material will be delivered by research staff, who will discuss the current scientific literature suggesting that NNS consumption is linked with obesity and cardiometabolic disease[26] and provide an overview of the emerging evidence that consumption in pregnancy/lactation may have unfavorable effects on infants' health.[5,27] Participants will also receive handouts to reinforce the counseling provided and support adherence to their assigned treatment. The research staff will also provide personalized counseling on NNS containing foods and beverages that should be avoided during the study, along with a list of foods and beverages containing NNS to avoid while enrolled in the study.

Research staff will emphasize that added sugars are not the best alternative to NNS, and that the participant should drink unsweetened water, sparkling water, flavored

waters with no added sweeteners, or unsweetened tea instead. The participant will also be provided with sample unsweetened beverages of their choice to take home and will receive bi-weekly deliveries of unsweetened beverages selected from a list of pre-specified offerings throughout the study. Participants will receive a text message to select a two-week supply of unsweetened beverages to be mailed to their home every two weeks. While only replacements for NNS-containing beverages (and not NNS-containing foods) will be provided by the study for logistical and budgetary reasons, participants assigned to NNS restriction will be instructed and counseled to avoid all NNS-containing foods and beverages throughout the study.

The control intervention (home safety) will be of comparable intensity to the NNS restriction intervention but will not include beverage deliveries. During the in-person visits, research staff will counsel the participants about best practices for home safety (pregnancy) and baby proofing (postpartum). Participants will also receive detailed handouts to take home, which will provide information about home safety and baby proofing. Research staff will educate participants about common causes of accidental death and/or injuries, with emphasis on general home safety (e.g., smoke detectors) during pregnancy and baby proofing (e.g., keeping medications out of reach) and infant safety (e.g., accidental drowning) after delivery. The intervention topics for each study visit are summarized in **Supplemental Table 2**. Additional resources and videos on home safety and babyproofing will be provided, and text messages with reminders about home safety, infant safety, and baby proofing will be sent to mothers each week.

Assignment of interventions

We will use a blocked randomization with a block size of six. Using a random number generator, the study statistician will create a predefined list of group assignments, ensuring each successive set of six women includes two assigned to Group 1, two to Group 2, and two to Group 3. This information about the blocking will be provided in a separate document that is unavailable to study staff enrolling participants or assigning interventions to reduce predictability. The design is open-label, with only outcome assessors blinded; it is essential for the research team to know the allocation to provide the correct intervention materials and relevant counseling and for the participants to be able to adhere to their treatment assignment.

Sample size

This study has two co-primary endpoints: infant adiposity (fat mass percent) at 6 months of age and maternal glucose tolerance (120-minute iAUC during the OGTT[28]) at 28 weeks gestation. To control the family-wise type I error rate at 5%, we will use a Bonferroni adjustment and test treatment effect on each co-primary endpoint using a significance level of 2.5%.

For the infant body adiposity outcome, we calculated that a sample size of 70 participants per treatment arm (210 total) would provide over 80% power to demonstrate a difference between the treatment groups using ANOVA with a significance level of 2.5%. The following assumptions were made for this power analysis: (1) infant body fat percentage for the control arm (Arm 1) is approximately 9.3%[29], body fat percentage for the NNS restriction during pregnancy and lactation arm (Arm 3) is 6.98% (25% lower than Arm 1), and body fat percentage for the NNS

restriction during lactation only arm (Arm 2) is the 8.14% (mean of Arm 1 and Arm 3; 12.5% lower than Arm 1); (2) the within arm variance is 15.21% (based on SD = 3.9%).[29] If there is a significant overall treatment effect for the infant adiposity outcome, a Benjamini–Hochberg step-down procedure will be used to control the false discovery rate for the multiple testing of three comparisons (Group 2 vs. Group 1, Group 3 vs. Group 1, and Group 2 vs. Group 3). If the lowest of the three p-values is less than 0.83% (2.5/3), it is considered statistically significant, and the second lowest p-value is considered statistically significant if less than 1.25% (2.5/2). If both are significant, then the third p-value is considered statistically significant if less than 2.5%. For the lowest significance level, 0.83%, we will have over 80% power to detect an effect size of at least Cohen's d = 0.60 (traditionally considered a medium effect size) for the corresponding pairwise comparison.

These sample size estimates are conservative because we plan to use a linear mixed effects ANCOVA model that will utilize repeated measures from the same participants. We will likely be able to detect even smaller group differences with our sample.

For the maternal glucose tolerance outcome, measured via iAUC during a 2 hour OGTT at 28 weeks gestation, having 70 participants in the NNS restriction during both pregnancy and lactation arm (Group 3) and 140 in the combined group of those in the control arm and those in the NNS restriction during lactation only arm (Groups 1 + 2) would provide 80% power to detect an effect size of Cohen's d = 0.45 (medium) using a two-sided t-test at a significance level of 2.5%.

To account for the high likelihood of breastfeeding failure/cessation prior to 6 months of age, the likelihood that some participants will experience pregnancy loss, the long duration of study participation, and to ensure that at least 210 mother-infant dyads complete the study, we conservatively plan for 35% attrition and will enroll 324 pregnant participants.

Participant Timeline

As shown in **Figure 1** and detailed below, the study will consist of a virtual screening visit and four in-person study visits (two during pregnancy and two postpartum) as well as monthly virtual consultations, twice-monthly unsweetened beverage deliveries (for NNS restriction group only), and weekly text message reminders throughout the study. Participants will also be contacted by phone to complete two 24-hour dietary recalls (one weekday, one weekend day) during the week prior to each in-person study visit.

Virtual screening visit

During the virtual screening visit, a research staff member will describe the study purpose and procedures, including the schedule of in-person visits, virtual consultations, beverage deliveries, phone calls, and text message reminders. Eligibility will be confirmed, informed consent will be obtained electronically using REDCap[25], and participants will be scheduled for a baseline in-person study visit, which will take place between 12 weeks and 18 weeks gestation.

Pregnancy study visits

During the week prior to the baseline visit (and at the three subsequent in-person visits), participants will complete two 24-hour dietary recalls by telephone. Participants who are unable to be reached or who cannot complete both dietary recalls by telephone prior to their in-person visit will complete a dietary recall during the in-person study visit to minimize missing data. The interviewer will use a multi-pass approach[30] to maximize the likelihood of accurate dietary recall and dietary data will be analyzed using the Nutrition Data System for Research (NDSR) software. Participants will also be asked to collect a stool sample within the 48 hours prior to their scheduled baseline study visit, using a sterile stool sample collection kit that will be mailed to them. Upon arriving for the baseline in-person study visit following a 10-hour fast, the participant will provide a spot urine sample and a blood sample will be collected by a trained phlebotomist to measure basal glucose and insulin. Additional plasma will be collected and stored for future analysis of other analytes of interest. Participants will bring the stool sample they collected during the 48 hours prior to the visit and provide it to the research staff. If the participant was unable to obtain a stool sample, they will be provided with a new collection tube (if needed) and asked to provide a sample within 48 hours of their visit.

Research staff will measure participants' height and weight, in duplicate, using an electronic scale (BWB-800S, Tanita, Portage, MI), stadiometer (Model PE-AIM-101, Perspective Enterprises), as well as bioelectrical impedance (InBody) and blood pressure. Following biospecimen collection and anthropometric measurements, participants will complete questionnaires about demographics (parental education, household income, eligibility for WIC or SNAP), food security,[31] physical activity,[32] food addiction symptoms,[33] stress,[34] sleep,[35] depression,[36] medical history, and antibiotic use

(type, dosage, duration), electronically using REDCap.[25] An interviewer-administered NNS intake questionnaire, developed by the study team and used in prior studies,[23,24] will be administered by the Research Assistant. For each NNS-containing food or beverage consumed, participants will be asked to provide detailed information about the type(s) and brand(s) of the NNS- containing products they consume, as well as the amount and frequency of consumption in the past week. This will allow us to identify specific NNS being consumed or avoided (per randomization) and explore potential differences in study outcomes by type of NNS.

Following collection of the baseline NNS intake data, participants will be randomly assigned to one of the three treatment groups and will receive counseling per their randomization, as described above. Participants will also be scheduled for their first virtual monthly consultation (~20 weeks) and will be provided with additional sample unsweetened beverages to take home (NSS restriction group only) in order to ensure they have replacement beverages until the beverage shipment arrives. Adherence will be assessed using monthly NNS intake questionnaires, which will be administered by a Research Assistant at each in-person and virtual study visit.

During the second in-person study visit at 28-30 weeks gestation, participants will complete a 75 g 2-hour OGTT after an overnight fast. Prior to beginning the OGTT, the research staff will measure the participant's weight, blood pressure and bioelectrical impedance, consistent with the procedures described above for the baseline visit, and the participant will be asked to provide a urine sample. An intravenous catheter will then be placed in an antecubital vein with the participant in the recumbent position. A blood sample will be collected at baseline (-10 min) and at 10-, 20-, 30-, 60-, 90-, and 120-

minutes following ingestion of a standard glucose/water mixture containing 75 g glucose (TRUTOL 75; Thermo Scientific, Middletown, VA) to allow for serial measurement of glucose and insulin. Venous blood samples will be collected into blood tubes for serum (serum separator tube) and plasma (K-EDTA and Li-Hep), centrifuged at 4°C, and plasma stored at -80°C until analysis. In the event we are unable to place the intravenous catheter or if it needs to be removed during the OGTT, we will collect capillary blood glucose via fingerstick to ensure we are able to measure our primary outcome (glucose iAUC).

Serial samples collected from each participant will be analyzed in duplicate within a single assay. Total and incremental area under the glucose (120 min) and insulin (120 min) response curves (AUC) will be calculated by the trapezoidal method.

In order to obtain additional information regarding glucose/insulin dynamics, the composite *whole-body insulin sensitivity index (WBISI)* will be calculated from basal glucose and insulin levels and values averaged over the 2-hour OGTT as 10,000/[sqrt(glucose0 x insulin0) x (mean glucose0-120 x mean insulin0-120)].[37] Lower WBISI indicates greater insulin resistance. We will also calculate the *homeostasis model assessment of insulin resistance* (HOMA-IR): [fasting insulin (uU/mL)] X [fasting glucose (mmol/L)]) / 22.5]. Higher HOMA-IR values indicate insulin resistance.

During the OGTT, participants will complete food security, physical activity, stress, sleep, depression, medical history, and antibiotic use questionnaires electronically using REDCap, analogous to the baseline visit. Consistent with the baseline visit and monthly virtual visits, the NNS intake questionnaire will then be administered by a research staff member. Participants will receive counseling in accordance with their randomization, as

well as handouts to reinforce the counseling provided and support adherence to their treatment assignment, as described above.

Following the final OGTT blood draw (120 min), the participant will be given a snack and/or water, as desired, and will schedule their 32-week virtual visit. The participant will also be provided with two sterile stool sample collection kits to collect their infants' stool during the 48 hours prior to the 1-month postpartum visit, as detailed below.

Virtual delivery check-in

As soon as possible following delivery, participants will attend a brief virtual visit, during which information about the participant's pregnancy and birth will be collected electronically using a medical history form and research staff will administer the NNS intake questionnaire. Following questionnaire completion, counseling will be provided in accordance with the participant's randomization and the 1-month postpartum study visit will be scheduled.

Postpartum study visits

The postpartum study visits will take place at 1-month and 6-months postpartum. During the day prior to each postpartum study visit, the mother will be asked to complete a 24-hour infant feeding log. Mothers will also be instructed to collect a sample of the infant's stool within the 48 hours to the visit, by transferring it directly from soiled diapers into two sterile stool collection tubes in the kits provided by the study team. One of the tubes will contain DNA/RNA shield (Zymo Research Corporation USA) that preserves the sample at room temperature for 30 days and the second tube will

contain DNA Genotek solution (Metabolon; Morrisville, NC, USA) that preserves the sample at room temperature for 7 days.

Upon arriving for the 1 month and 6-month postpartum study visits, the participant will be asked to provide a sample of their urine along with the stool samples collected from their infant prior to the study visit. All stool samples will be subsequently be stored at -80°C after collection until metagenomics and metabolomics analysis, as detailed below. The mother will then provide a fasted blood sample, collected by a trained phlebotomist, and subsequently complete surveys and the research-staff administered NNS intake questionnaire, analogous to at the prior in-person visits. The mother's weight and waist circumference will be measured, in duplicate.

The infant's medical history, antibiotic use, and feeding practices will be reported by the mother. Infant length will be measured using a length board and weight will be measured without clothes using an infant scale. The infant will then be placed without clothing in the PeaPod[38] for approximately two minutes to measure body volume using air displacement plethysmography. As applicable, the infant's hair will be kept flat using a cap. Body composition will be measured using PeaPod software 3.0.1 (Cosmed, Concord, CA, USA), which calculates body density from body weight, body volume, and body composition based on the fat-free mass density values predicted by the software. The PeaPod is simple to operate, highly accurate when compared to reference techniques,[39] and the entire procedure takes approximately 5 minutes from start to finish.

Research staff will then provide intervention counseling in accordance with the participant's randomization (except for at the 6-month postpartum visit) and the

participant will be provided with handouts to reinforce the content. As applicable (i.e., at the 1-month postpartum visit), the participant will also schedule their next virtual visit (2 months postpartum) and those in the NNS restriction groups (Group 2 or 3) will also order their next shipment of unsweetened beverages.

Monthly virtual consultations

During the monthly virtual consultations, participants will complete a medical history questionnaire electronically using REDCap and a research staff member will administer the NNS intake questionnaire, analogous to during the in-person visits. Intervention counseling will be conducted, per the participant's assigned treatment, and accompanying handouts to reinforce the intervention content will be provided electronically. During the monthly virtual consultations postpartum, information on infant feeding practices will also be obtained to determine whether the infant is fed mothers' own milk, and therefore exposed to NNS consumed by the mother, and/or if the infant may be exposed to NNS in foods and beverages they may consume.

Data management

All study data will be entered and stored in REDCap. Only members of the study team will have access to the study database and data files. Data will be collected, managed, and stored with unique identifiers using the web-based, password-protected REDCap application, which provides a validated data entry interface, audit trails, and automated export procedures. Customized data collection forms and a study-specific

data dictionary will be used. Data range and logic checks will ensure accuracy. Any loss of samples or data will be reported to the IRB.

All source documents will be completed in a neat, legible manner to ensure accurate entry of data. Hardcopies of the study visit worksheets will serve as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents will be consistent with the data recorded on the source documents. Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will also be entered into REDCap. The data system includes password protection. Plans for assessment and collection of outcome data include regular training of assessors to ensure consistency and accuracy of measurements. Duplicate measurements will be taken for weight, height, and blood pressure to enhance data quality and minimize measurement errors.

Laboratory Analyses

Glucose and insulin

Blood glucose will be measured via point-of-care analyzer (HemoCue Glucose 201; Beckman Coulter, Inc., Brea, CA, USA). Plasma insulin (Sigma Aldrich; MA, USA) will be measured using colorimetric assays, per manufacturer instructions.

Urinary NNS

Sucralose, ace-k, saccharin, and stevia will be measured using LC-MS in Dr. Walter's lab at NIDDK. Consistent with our prior studies,[3,4,40-43] analyses will be performed with an Acquity I-Class UPLC (Waters, Milford, MA) coupled with a Q-Exactive MS (Thermo Scientific, Waltham, MA). While aspartame is not measurable in biospecimens due to rapid degradation following ingestion, we will be able to objectively measure the other NNS and will be able to estimate aspartame intake using the detailed dietary data collected.

Gut microbiota

DNA sequencing will be performed at the GWU Genomics Core (RRID:SCR_027546). DNA extraction will be performed using the Zymo Quick-DNA Fecal/Soil Microbe DNA Miniprep kit. Sequencing libraries will be prepared using the Nextera XT kit and sequenced using shotgun metagenomics sequencing using the NextSeq 2000 High-Output 300 cycle kits (Illumina) with a ZymoBIOMICS Gut Microbiome Standard for benchmarking and validation of the NGS microbiome workflow. For quality control, whole metagenomics shotgun sequencing reads from these samples will be filtered and trimmed to eliminate low-quality reads using FASTQC/MultiQC.[44] Then, quality reads will be mapped to the human genome (hg38) to partition human reads from the remaining reads. The remaining reads will be classified using PathoScope2[45] and the resulting microbial diversity will be functionally profiled by HUMAnN2.[46] Variability of microbial communities will be assessed at the microbial diversity level alpha (e.g., Shannon index) and beta diversities using Bray-Curtis distances and omeClust,[47] at the individual species level. Principal coordinate analyses (PCoA) will be used to display the samples in PCoA space. For multivariate analyses, we

will test for significant community structure by group using the omnibus PERMANOVA (in the R package vegan), a nonparametric test that accounts for the correlated structure in the distance matrix. We will use MaAsLin3 to test for significant associations between microbial diversity and abundance and clinical metadata.[48]

Stool metabolomics

Frozen stool samples will be lyophilized and resuspended at a 50:1 (50 µl deionized water per 1 mg of feces) ratio for homogenization. Homogenates will be subjected to automated biochemical extraction and analysis by liquid chromatography and high-resolution tandem mass spectrometry (MS/MS) on Metabolon's Global Platform. Raw data will be extracted and peaks will be identified and processed using MassSight software. Metabolites will be identified by comparison to library entries of purified standards or recurrent unknown entities. We will use profiling techniques to measure a comprehensive range of metabolites (e.g., lipids, free fatty acids, bile acids, lipid mediators, sugars, organic acids, purines, pyrimidines, and central metabolites), including annotated and unannotated metabolites. Three types of controls will be included: a pool of small portions of each experimental sample, serving as a technical replicate throughout the platform run; extracted water samples (process blanks); and a mixture of standards spiked into every analyzed sample, allowing for monitoring of instrument performance and drifts in metabolites descriptors (m/z and retention time). We will perform error correction, normalization, drift correction, and quality control techniques on the metabolite data, including data cleaning, data curation, and drift correction for enhanced interpretation. Clinical and metabolomics data will be analyzed to identify metabolites of

interest and key clinical features associated with significant changes in metabolomics between the treatment groups and over time.

Study outcomes

The co-primary outcomes are infants' adiposity (mean fat mass percent) at 6 months of age, and mothers' mean incremental area under the 120-minute glucose response curves during a 75 g OGTT at 28 weeks gestation. These primary outcomes were selected because infants' adiposity is predictive of future obesity risk and aberrant glucose control in pregnancy is highly associated with increased infant adiposity and future childhood obesity risk. The primary, secondary and exploratory study outcomes are described in **Table 1**.

Statistical methods

An intention-to-treat approach will be used for the main analysis, along with per-protocol analysis removing non-adherent participants. For the co-primary infant adiposity outcome, we will employ a linear mixed effects model with infant fat mass percent at 1 month and 6 months of age as the outcome. Predictors in the model will include treatment group, time, and treatment group by time interaction. The model will also include an infant-specific random intercept to account for multiple observations from the same infant. Model-based estimates will be used to test whether the means differ according to treatment group and/or over time. If an interaction is statistically significant at the 2.5% level, stratified analyses will be conducted to examine group differences at each time point and time-trends within each group. If the group-by-time

interaction is not statistically significant, overall group and time comparisons will be performed at the 2.5% significance level. If there is evidence of a statistically significant treatment effect, the Benjamini–Hochberg step-down procedure will be used to control the false discovery rate (FDR) at 2.5% for the test of the pairwise comparisons.

For the co-primary maternal glucose tolerance outcome measured as iAUC at 120 minutes after the OGTT at 28-weeks' gestation, we will employ a two-sample t-test to compare iAUC between the NNS restriction during both pregnancy and lactation group (Group 3) and the combined group of those in the control arm and those in the NNS restriction during lactation only arm (Groups 1 and 2). If necessary, transformations (e.g., log) of the outcomes will be made to satisfy model assumptions. The hypothesis testing strategy and corresponding decision rules for the co-primary outcomes are summarized in **Table 2**. Analyses for secondary outcomes will follow approaches analogous to those used for the co-primary outcomes.

Exploratory analyses will assess whether treatment effect varies by other factors (i.e., effect modification) including: race/ethnicity, maternal stress, maternal or infant antibiotic use, feedings per day, and types of NNS habitually consumed. To evaluate effect modification for the infant adiposity outcome, we will extend the primary linear mixed effects model to include the potential effect modifier, along with its two-way interactions with treatment group and time, and the three-way interaction among the potential effect modifier, treatment group, and time. To evaluate effect modification for maternal glucose tolerance, we will fit linear regression models with iAUC at 120 minutes after OGTT at 28 weeks' gestation as the outcome and terms for treatment group, the potential effect modifier, and their interaction.

We will also conduct mediation analyses to determine the effect of treatment on infant adiposity is mediated by maternal blood glucose, inflammatory biomarkers, or specific gut microbes or metabolites.

Information on reasons for missing data and study discontinuation will be collected when available. Patterns and rates of missingness will be summarized and we will investigate associations between missingness and baseline or post-baseline variables to assess the reasonableness of missing at random (MAR) assumptions. Primary analyses will be conducted under the MAR framework, and we will employ methods that are valid under this assumption. If more than 5% of the outcome data for a given analysis are missing, we will conduct multiple imputation and compare results with complete case analysis. In this case, we will also conduct sensitivity analyses to assess robustness of our conclusions to departures from the MAR assumption, including analyses under various missing not at random assumptions.

Oversight and monitoring

Adverse event (AE) monitoring and safety oversight for the trial will be led by the Principal Investigator (PI) in collaboration with the co-investigators. The research team will meet regularly to review participant safety and address any reported events. For the purpose of this study, an adverse event is defined as any untoward medical occurrence in a participant, including abnormal signs, symptoms, or diseases related to study participation. Serious adverse events (SAEs) are classified as those that result in death, are life-threatening, require hospitalization or prolong an existing hospitalization, cause

significant disability, result in congenital anomalies, or jeopardizes health and require intervention to prevent these outcomes.

All AEs will be assessed for severity, categorized as mild, moderate, or severe, and evaluated for their relationship to the study procedures. A trained clinician will use temporal association and clinical judgment to determine causality, classifying events as unrelated, possibly, probably, or definitely related to the protocol. Each participant will be evaluated for AEs throughout the study, and any AE will be documented in study records. Documentation will include the nature of the event, its timing, severity, attribution, and any corrective actions taken.

Severe or unexpected adverse events will be reported immediately. Reports will be submitted to the Institutional Review Board (IRB) within 48 hours and to the Data and Safety Monitoring Board (DSMB) within 24 hours. All adverse events will also be summarized in annual reports to the IRB and the NIH, detailing their frequency, severity, and any subsequent modifications to the study protocol or consent forms.

The PI will review all reported AEs, assess their impact on the study, and determine whether any modifications to the protocol or additional safety measures are needed. The PI will notify co-investigators, the IRB, and the DSMB of any serious events and ensure prompt action to mitigate risks. Regular safety assessments and detailed reporting will ensure participant well-being and maintain the integrity of the trial.

The DSMB will oversee the trial to ensure participant safety and the integrity of the data. The DSMB will be composed of four independent members with relevant expertise, including a biostatistician, an obstetrician-gynecologist, a neonatologist, and a clinician scientist. Members will provide written declarations of no conflict of interest.

The DSMB will operate independently from the investigators and the sponsor, with a charter outlining its responsibilities and governance. Details of the DSMB charter will be provided upon request.

The primary responsibilities of the DSMB include reviewing participant safety, assessing data quality, and monitoring trial progress. The DSMB will meet twice annually during active data collection to review interim safety reports, participant recruitment and retention rates, and data quality assessments. The DSMB will also consider external factors, such as new scientific findings, that may affect participant safety or the trial's relevance. No formal interim analyses are planned for this trial. However, the DSMB will have access to safety and performance data throughout the study and will recommend modifications or termination of the trial if necessary. The DSMB may recommend modifying or stopping the study if safety concerns arise, if the study is performing inadequately, or if the study objectives have already been attained. Final decisions regarding trial modifications or termination will be made by the PI in consultation with the DSMB and IRB.

Regular self-audits will be conducted to ensure adherence to the study protocol and regulatory requirements. The research team will meet bi-weekly to review trial progress and resolve any identified issues. Additionally, supervised visits will be conducted to verify compliance with the protocol and to address challenges in real time. Independent audits by DSMB will occur twice annually during active data collection. These audits will assess data quality, participant recruitment and retention, adherence to ethical standards, and overall study progress.

Dissemination plans

The trial was registered on ClinicalTrials.gov (NCT06548828) prior to enrollment of the first participant. Trial results will be shared through peer-reviewed publications and presentations at local, national, and/or international conferences, and registration on ClinicalTrials.gov. Publications will follow CONSORT guidelines, and authorship assignment will align with International Committee of Medical Journal Editors (ICMJE) recommendations.

Discussion

To our knowledge, the SweetPea trial is the first RCT to evaluate the impacts of NNS consumption during pregnancy and/or lactation on maternal and infant health outcomes. The RCT design, combined with robust biospecimen, dietary, and questionnaire data and repeated measures throughout both pregnancy and postpartum, and from both the mother and the infant, will allow us to address significant gaps in the existing literature. To date, evidence on the effects of intergenerational NNS exposure has been comprised entirely of observational studies that often face issues of reverse causality and residual confounding,[5] with the only experimental studies conducted in animal models.[27] Findings of this trial will therefore improve understanding of whether NNS consumption affects infant body composition, maternal glycemia, and the infants' gut microbiome and fecal metabolites, or if results of prior epidemiologic studies may simply be explained by reverse causality and residual confounding.

Most prior studies have focused only on offspring BMI z-score rather than adiposity or other relevant outcomes such as maternal blood glucose and infants' gut

microbiome and fecal metabolites. Measurement of infant body composition using the PEAPOD, a highly accurate and noninvasive method of determining infant adiposity and body composition will provide a more accurate reflection of obesity risk than BMI z-score. Another key strength of the study design is the ability to conduct integrated analyses of infant adiposity, gut microbiome, fecal metabolites, which will provide important mechanistic insight into the relationship between NNS exposure and infant health. Infant data collection at both 1 month and 6 months of age will also allow us to examine effects of *in utero* and/or postnatal NNS exposure longitudinally during infancy.

A key limitation of the study design is the relatively short-term follow-up of infants, as more than 6 months may be required to observe effects of NNS restriction. We also will not have OGTT data at baseline, as conducting an OGTT at enrollment would be burdensome for participants and may be difficult due to nausea in early pregnancy. However, we will collect a fasting blood sample at baseline, which will provide information on mothers' blood glucose regulation prior to randomization.

Despite these limitations and given the robust measures on both mothers and their infants, this trial will provide urgently needed information to determine whether NNS restriction improves both maternal and infant metabolic health. The current investigation is critical given the widespread and increasing use of NNS among pregnant and lactating women and the lack of evidence-based guidelines for their consumption during these critical developmental periods.

Trial status: The trial is actively recruiting. We report protocol version 1.11 dated November 10, 2025. Recruitment started in August 2024, with the first participant

enrolled on September 14th, 2024. At the time of submission of this manuscript on March 20th 2026, 112 participants have been randomized. Recruitment is ongoing and should be completed by February 28th, 2027. The last participant is expected to complete the study by February 28th, 2028.

Abbreviations

ADI - Acceptable daily intake

AE- Adverse event

ANCOVA- Analysis of covariance

AUC- Area under the curve

BMI- Body mass index

C- Celsius

Co-I- Co-investigator

CRF- Case report form

CRP- C reactive protein

DSMB- Data and safety monitoring board

eCRF- Electronic case report form

g- Gram

GDM- Gestational diabetes mellitus

GW- George Washington University

GWG- Gestational weight gain

HIPAA- Health insurance portability and accountability act

HOMA-IR- Homeostasis model assessment of insulin resistance

IPAQ- International Physical Activity Questionnaire

IRB- Institutional review board

L- Liter

LC-MS- Liquid chromatography-mass spectrometry

LDL- Low density lipoprotein

mL- Milliliter

mmol- Millimole

MS- Mass spectrometry

NDSR- Nutrition Data System for Research

NICHD- National Institute of Child Health and Development

NIH- National Institutes of Health

NNS- Nonnutritive sweeteners

OGTT- Oral glucose tolerance test

PI- Principal investigator

RA- Research assistant

RCT- Randomized controlled trial

REDCap- Research electronic data capture

SAE- Serious adverse event

UP- Unanticipated problem

WBISI- Whole body insulin sensitivity index

Declarations

Availability of data and materials: All sequence and metadata will be made available in a publicly available repository, such as NCBI's Short Read Archive and BioProject descriptions. The sequence data and metadata will be made available at the time of the first publication or by the end of the funding period for the grant, whichever comes first. Relevant clinical data summaries (de-identified) and metabolomics data will be made available as supplementary materials to published work at the journal's website and be available from the PI upon request.

Ethics approval and consent to participate: The Institutional Review Board (IRB) at the George Washington University reviewed and approved the study protocol (NCR235110). All participants provided informed consent electronically via REDCap prior to beginning the study procedures.

Consent for publication: Not applicable. No individual participant data are presented here or will be presented when reporting the trial results.

Competing interests: The Principal Investigator and study team declare no financial or competing interests related to this trial. Conflict of interest policies established by The George Washington University will be followed, and any conflicts arising during the study will be disclosed and managed appropriately.

Funding: This trial is supported by the National Institute of Child Health and Development (NICHD) at the National Institutes of Health (R01HD107427A1, PI: Sylvetsky).

Authors' contributions: AS, MB, ES, AC, and MG designed the study protocol. JV, JM, KC, AR, PW, and COS participated in the development of the research. VB, MI, and NF have played a key role in the collection and management of the study data. AS, VB, and MI drafted the manuscript. All authors contributed to editing the manuscript and approved of the final version.

Acknowledgements: The authors are grateful to the following staff who have contributed to the development and implementation of the study protocol described herein: MI, NL, GG, AB, DN, and NS recruited prospective participants from clinics. VB, MI, PP, KSC, and EM screened participants, determined eligibility, and administered consent. VB, MI, PP, XW, ACC, KSC, NF, AB, AN and AB randomized participants and conducted in-person study visits. PP, XW, ACC, KSC and EM administered dietary recalls. VB, MI, PP, XW, ACC, KSC and EM conducted virtual study visits. JB, SR, AVV, AS, NE & NT performed blood draws during in-person study visits. VB, MI, UM, and AC coordinated study logistics. The authors also thank the members of the Data Safety of Monitoring Board for their support in monitoring the trial and providing constructive feedback. Finally, the authors are grateful to the leadership and staff at the GW Medical Faculty Associates for allowing us to engage participants in the Ob/Gyn, Maternal Fetal Medicine, and Midwifery clinics and helping to share information about the study to their patient population.

References

1. Sylvetsky AC, Figueroa J, Rother KI, Goran MI, Welsh JA. Trends in Low-Calorie Sweetener Consumption Among Pregnant Women in the United States. *Curr Dev Nutr*. Apr 2019;3(4):nzz004. doi:10.1093/cdn/nzz004
2. Huang Q, Murphy J, Smith ER, Sylvetsky AC. Diet Beverage Intake during Lactation and Associations with Infant Outcomes in the Infant Feeding Practices Study II. *Nutrients*. Sep 10 2021;13(9)doi:10.3390/nu13093154
3. Halasa BC, Sylvetsky AC, Conway EM, et al. Non-Nutritive Sweeteners in Human Amniotic Fluid and Cord Blood: Evidence of Transplacental Fetal Exposure. *Am J Perinatol*. Sep 9 2021;doi:10.1055/s-0041-1735555
4. Rother KI, Sylvetsky AC, Walter PJ, Garraffo HM, Fields DA. Pharmacokinetics of Sucralose and Acesulfame-Potassium in Breast Milk Following Ingestion of Diet Soda. *J Pediatr Gastroenterol Nutr*. Mar 2018;66(3):466–470. doi:10.1097/MPG.0000000000001817
5. Reid AE, Chauhan BF, Rabbani R, et al. Early Exposure to Nonnutritive Sweeteners and Long-term Metabolic Health: A Systematic Review. *Pediatrics*. Mar 2016;137(3):e20153603. doi:10.1542/peds.2015-3603
6. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate of low-calorie sweeteners. *Nutr Rev*. Nov 2016;74(11):670–689. doi:10.1093/nutrit/nuw032
7. Olivier-Van Stichelen S, Rother KI, Hanover JA. Maternal Exposure to Non-nutritive Sweeteners Impacts Progeny's Metabolism and Microbiome. *Front Microbiol*. 2019;10:1360. doi:10.3389/fmicb.2019.01360
8. Plows JF, Morton-Jones J, Bridge-Comer PE, et al. Consumption of the Artificial Sweetener Acesulfame Potassium throughout Pregnancy Induces Glucose Intolerance and Adipose Tissue Dysfunction in Mice. *J Nutr*. Jul 1 2020;150(7):1773–1781. doi:10.1093/jn/nxaa106
9. Azad MB, Sharma AK, de Souza RJ, et al. Association Between Artificially Sweetened Beverage Consumption During Pregnancy and Infant Body Mass Index. *JAMA Pediatr*. Jul 01 2016;170(7):662–70. doi:10.1001/jamapediatrics.2016.0301
10. Archibald AJ, Dolinsky VW, Azad MB. Early-Life Exposure to Non-Nutritive Sweeteners and the Developmental Origins of Childhood Obesity: Global Evidence from Human and Rodent Studies. *Nutrients*. Feb 10 2018;10(2)doi:10.3390/nu10020194
11. Zhu Y, Olsen SF, Mendola P, et al. Maternal consumption of artificially sweetened beverages during pregnancy, and offspring growth through 7 years of age: a prospective cohort study. *Int J Epidemiol*. Jun 06 2017;doi:10.1093/ije/dyx095
12. Pereira TJ, Moyce BL, Kereliuk SM, Dolinsky VW. Influence of maternal overnutrition and gestational diabetes on the programming of metabolic health outcomes in the offspring: experimental evidence. *Biochem Cell Biol*. Oct 2015;93(5):438–51. doi:10.1139/bcb-2014-0141
13. Plows JF, Aris IM, Rifas-Shiman SL, Goran MI, Oken E. Associations of maternal non-nutritive sweetener intake during pregnancy with offspring body mass index and body fat from birth to adolescence. *Int J Obes (Lond)*. Jan 2022;46(1):186–193. doi:10.1038/s41366-021-00897-0
14. Zhang GH, Chen ML, Liu SS, et al. Effects of mother's dietary exposure to acesulfame-K in Pregnancy or lactation on the adult offspring's sweet preference. *Chem Senses*. Nov 2011;36(9):763–70. doi:10.1093/chemse/bjr050
15. Suez J, Korem T, Zeevi D, et al. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature*. Sep 17 2014;514(7521):181–6. doi:10.1038/nature13793
16. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. The artificial sweetener acesulfame potassium affects the gut microbiome and body weight gain in CD-1 mice. *PLoS One*. 2017;12(6):e0178426. doi:10.1371/journal.pone.0178426

17. Bian X, Chi L, Gao B, Tu P, Ru H, Lu K. Gut Microbiome Response to Sucralose and Its Potential Role in Inducing Liver Inflammation in Mice. *Front Physiol*. 2017;8:487. doi:10.3389/fphys.2017.00487
18. Bian X, Tu P, Chi L, Gao B, Ru H, Lu K. Saccharin induced liver inflammation in mice by altering the gut microbiota and its metabolic functions. *Food Chem Toxicol*. Sep 2017;107(Pt B):530–539. doi:10.1016/j.fct.2017.04.045
19. Suez J, Cohen Y, Valdes-Mas R, et al. Personalized microbiome-driven effects of non-nutritive sweeteners on human glucose tolerance. *Cell*. Sep 1 2022;185(18):3307–3328 e19. doi:10.1016/j.cell.2022.07.016
20. Hansen TH, Gobel RJ, Hansen T, Pedersen O. The gut microbiome in cardio-metabolic health. *Genome Med*. 2015;7(1):33. doi:10.1186/s13073-015-0157-z
21. Russell C, Baker P, Grimes C, Lindberg R, Lawrence MA. Global trends in added sugars and non-nutritive sweetener use in the packaged food supply: drivers and implications for public health. *Public Health Nutr*. May 2023;26(5):952–964. doi:10.1017/S1368980022001598
22. Yajnik CS, Deshmukh US. Maternal nutrition, intrauterine programming and consequential risks in the offspring. *Rev Endocr Metab Disord*. Sep 2008;9(3):203–11. doi:10.1007/s11154-008-9087-z
23. Sylvetsky AC, Kuttamperoor JT, Langevin B, et al. Intergenerational transmission of sucralose and acesulfame-potassium from mothers to their infants via human milk: a pharmacokinetic study. *Am J Clin Nutr*. Oct 2024;120(4):846–853. doi:10.1016/j.ajcnut.2024.08.001
24. Sylvetsky AC, Moore HR, Zhu X, et al. Effects of Low-Calorie Sweetener Restriction on Glycemic Variability and Cardiometabolic Health in Children with Type 1 Diabetes: Findings of a Pilot and Feasibility Study. *Nutrients*. Sep 05 2023;15(18)doi:10.3390/nu15183867
25. Harris PA, Taylor R, Thielke R, Payne J, Gonzalez N, Conde JG. Research electronic data capture (REDCap)--a metadata-driven methodology and workflow process for providing translational research informatics support. *J Biomed Inform*. Apr 2009;42(2):377–81. doi:10.1016/j.jbi.2008.08.010
26. World Health Organization. *Use of non-sugar sweeteners: WHO guideline.* 2023. https://www.who.int/publications/i/item/9789240073616
27. Kearns ML, Reynolds CM. The impact of non-nutritive sweeteners on fertility, maternal and child health outcomes: a review of human and animal studies. *Proc Nutr Soc*. Dec 2024;83(4):280–292. doi:10.1017/S0029665124000168
28. Sakaguchi K, Takeda K, Maeda M, et al. Glucose area under the curve during oral glucose tolerance test as an index of glucose intolerance. *Diabetol Int*. Mar 2016;7(1):53–58. doi:10.1007/s13340-015-0212-4
29. Sauder KA, Perng W, Palumbo MP, et al. Fat Mass Accretion from Birth to 5 Years and Metabolic Homeostasis in Childhood: the Healthy Start Study. *J Clin Endocrinol Metab*. May 13 2021;106(6):1684–1691. doi:10.1210/clinem/dgab115
30. U.S. Department of Agriculture ARS. AMPM - USDA Automated Multiple-Pass Method. Accessed October 6th, 2016. https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/ampm-usda-automated-multiple-pass-method/
31. Economic Research Service USDoA. U.S. Household Food Security Survey Module: Six-Item Short Form https://www.ers.usda.gov/topics/food-nutrition-assistance/food-security-in-the-u-s/survey-tools/
32. Lee PH, Macfarlane DJ, Lam TH, Stewart SM. Validity of the International Physical Activity Questionnaire Short Form (IPAQ-SF): a systematic review. *Int J Behav Nutr Phys Act*. 2011;8:115. doi:10.1186/1479-5868-8-115
33. Gearhardt AN, Corbin WR, Brownell KD. Development of the Yale Food Addiction Scale Version 2.0. *Psychol Addict Behav*. Feb 2016;30(1):113–21. doi:10.1037/adb0000136

34. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav*. Dec 1983;24(4):385–96.
35. Buysse DJ, Reynolds CF, 3rd, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res*. May 1989;28(2):193–213.
36. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry*. Jun 1987;150:782–6. doi:10.1192/bjp.150.6.782
37. Henriquez S, Jara N, Bunout D, et al. Variability of formulas to assess insulin sensitivity and their association with the Matsuda index. *Nutr Hosp*. Sep–Oct 2013;28(5):1594–8. doi:10.3305/nh.2013.28.5.6512
38. Urlando A, Dempster P, Aitkens S. A new air displacement plethysmograph for the measurement of body composition in infants. *Pediatr Res*. Mar 2003;53(3):486–92. doi:10.1203/01.PDR.0000049669.74793.E3
39. Sainz RD, Urlando A. Evaluation of a new pediatric air-displacement plethysmograph for body-composition assessment by means of chemical analysis of bovine tissue phantoms. *Am J Clin Nutr*. Feb 2003;77(2):364–70. doi:10.1093/ajcn/77.2.364
40. Sylvetsky AC, Sen S, Merkel P, et al. Consumption of Diet Soda Sweetened with Sucralose and Acesulfame-Potassium Alters Inflammatory Transcriptome Pathways in Females with Overweight and Obesity. *Mol Nutr Food Res*. Jun 2020;64(11):e1901166. doi:10.1002/mnfr.201901166
41. Sylvetsky AC, Gardner AL, Bauman V, et al. Nonnutritive Sweeteners in Breast Milk. *J Toxicol Environ Health A*. 2015;78(16):1029–32. doi:10.1080/15287394.2015.1053646
42. Sylvetsky AC, Bauman V, Blau JE, Garraffo HM, Walter PJ, Rother KI. Plasma concentrations of sucralose in children and adults. *Toxicological and Environmental Chemistry*. 2016:1–8.
43. Sylvetsky AC, Walter PJ, Garraffo HM, Robien K, Rother KI. Widespread sucralose exposure in a randomized clinical trial in healthy young adults. *Am J Clin Nutr*. Feb 22 2017;doi:10.3945/ajcn.116.144402
44. Ewels P, Magnusson M, Lundin S, Kaller M. MultiQC: summarize analysis results for multiple tools and samples in a single report. *Bioinformatics*. Oct 1 2016;32(19):3047–8. doi:10.1093/bioinformatics/btw354
45. Hong C, Manimaran S, Shen Y, et al. PathoScope 2.0: a complete computational framework for strain identification in environmental or clinical sequencing samples. *Microbiome*. 2014;2:33. doi:10.1186/2049-2618-2-33
46. Franzosa EA, McIver LJ, Rahnavard G, et al. Species-level functional profiling of metagenomes and metatranscriptomes. *Nat Methods*. Nov 2018;15(11):962–968. doi:10.1038/s41592-018-0176-y
47. Rahnavard A, Chatterjee S, Sayoldin B, Crandall KA, Tekola-Ayele F, Mallick H. Omics community detection using multi-resolution clustering. *Bioinformatics*. May 11 2021;37(20):3588–94. doi:10.1093/bioinformatics/btab317
48. Nickols WA, Kuntz T, Shen J, et al. MaAsLin 3: refining and extending generalized multivariable linear models for meta-omic association discovery. *Nat Methods*. Mar 2026;23(3):554–564. doi:10.1038/s41592-025-02923-9

Table 1. Primary, Secondary and Exploratory Outcomes in the SweetPea Trial

Outcome	Type	Definition	Time period
Maternal outcomes			
Glucose tolerance	Primary	Incremental area under the 120-minute glucose response curves following ingestion of 75 g glucose	28-30 weeks gestation
Fasting blood glucose	Secondary	Blood glucose following a 10 hour fast	28-30 weeks gestation
Blood glucose at 60 minutes	Secondary	Blood glucose 60 minutes following ingestion of 75g glucose	28-30 weeks gestation
Blood glucose at 120 minutes	Secondary	Blood glucose 120 minutes following ingestion of 75g glucose	28-30 weeks gestation
Composite whole-body insulin sensitivity index (WBISI)	Secondary	Composite whole-body insulin sensitivity index (WBISI) =10,000/[sqrt(glucose0 x insulin0) x (mean glucose0-120 x mean insulin0-120)]	28-30 weeks gestation
Homeostasis model assessment of insulin resistance (HOMA-IR)	Secondary	Homeostasis model assessment of insulin resistance (HOMA-IR) = [(fasting insulin X fasting glucose)/dividing by 22.5]	28-30 weeks gestation
Energy intake	Secondary	Mean energy intake from two dietary recalls	28-30 weeks gestation, 1 month postpartum, 6 months postpartum
Total gestational weight gain	Exploratory	Self-reported body weight at delivery minus mothers' self-reported pre-pregnancy body weight	Delivery

Infant Outcomes			
Adiposity	Primary, secondary	Fat mass percentage	1 month postpartum (secondary), 6 months postpartum (primary)
Gestational age	Secondary	Gestational age at birth	Delivery
Birth weight	Secondary	Weight at time of birth	Delivery
Birth weight for gestational age	Secondary	Infant's birthweight based on their gestational age	Delivery
Weight for age	Secondary	Infant's weight based on their age, using WHO growth charts	1 month, 6 months
Gut microbiome composition	Exploratory	Relative abundance of Firmicutes, *Akkermensia*, Bifidobacterium, Lactobaccilus, and Proteobacteria	1 month, 6 months
Fecal metabolites	Exploratory	Metabolites including lipids, free fatty acids, bile acids, lipid mediators, sugars, organic acids, purines, and pyrimidines.	1 month, 6 months

Table 2. PRO EDI Participant Characteristics Table

Characteristics	**The people we would expect to see included**
Age	Eligibility requires age 18–45; exact age will be collected at baseline.
Sex	100% female (pregnant individuals).
Gender	Gender identity not collected.
Ethnicity/Race	Ethnicity/Race will be collected via baseline demographic questionnaire.
Socioeconomic Position	Socioeconomic indicators collected at baseline include parental education, household income, WIC/SNAP eligibility, and food security.
Geographic Context	Urban U.S. setting (Washington, D.C.); participants reside in the DC metro region.
Disability Status	Not collected.
Language / Literacy	Eligibility requires ability to read English at a 5th-grade level.
Other Relevant EDI Factors	Pregnancy-specific characteristics collected at baseline include gestational age, breastfeeding intention, parity, medical history, stress, sleep, depression, and antibiotic use.

Table 3. Hypothesis testing strategy and decision rules for co-primary outcomes.

Outcome	Step	Analysis/Test	Significance Level	Decision Rule/Next Step
Infant adiposity	1	Test treatment group x time interaction (in linear mixed effect model)	2.5%	If significant, proceed to Step 2a; otherwise proceed to Step 2b.
	2a	Stratified analyses (assess treatment group differences at each time and time trends within each group)	2.5% (use B-H to control FDR for pairwise comparisons)	Interpret stratified effects.
	2b	Main effects of treatment group and time	2.5%	If treatment effect is significant, proceed to Step 3.
	3	Pairwise treatment group comparisons	2.5% (use B-H to control FDR for pairwise comparisons)	Interpret any treatment group differences.
Maternal glucose tolerance	1	Two-sample t-test (treatment Group 3 vs. Groups 1 and 2 combined)	2.5%	Interpret group difference if present.

B-H: Benjamini-Hochberg
FDR: false discovery rate

Figure 1. Participant timeline: Schedule of enrollment, interventions, and assessments

	TRIAL PERIOD													
	Enrollment		Post-randomization											Close-out
TIMEPOINT	*Pre-screen*	12-18 weeks	*20 weeks ± 1 week*	*24 weeks ± 1 week*	*28 weeks ± 1 week*	*32 weeks ± 1 week*	*36 weeks ± 1 week*	*Delivery*	*1 mo. PP ± 1 week*	*2 mo. PP ± 1 week*	*3 mo. PP ± 1 week*	*4 mo. PP ± 1 week*	*5 mo. PP ± 1 week*	*6 mo. PP ± 1 week*
ENROLLMENT:		X												
Eligibility screen	X													
Informed consent	X													
Randomization		X												
INTERVENTION/ COMPARATOR:														
NNS reduction in pregnancy + lactation		X	→	→	→	→	→	→	→	→	→	→	→	X
NNS reduction in lactation only		X	→	→	→	→	→	X	→	→	→	→	→	X
No NNS reduction (control)		X	→	→	→	→	→	→	→	→	→	→	→	X
ASSESSMENTS:	**Maternal Assessments**													
NNS screener	X													
Pregnancy confirmation	X													
24-hour diet recalls (in duplicate)		X			X				X					X
Demographic information		X												

Obstetric, medical, and family history		X												
Clinical information pertaining to current pregnancy								X						
Maternal height		X												
TIMEPOINT	***Pre-screen***	**12-18 weeks**	***20 weeks ± 1 week***	***24 weeks ± 1 week***	***28 weeks ± 1 week***	***32 weeks ± 1 week***	***36 weeks ± 1 week***	***Delivery***	***1 mo. PP ± 1 week***	***2 mo. PP ± 1 week***	***3 mo. PP ± 1 week***	***4 mo. PP ± 1 week***	***5 mo. PP ± 1 week***	***6 mo. PP ± 1 week***
Pre-pregnancy weight		X												
Maternal weight		X			X				X					X
GWG velocity		X			X									
Waist circumference									X					X
Food Security		X			X				X					X
Food Addiction Scale		X			X				X					X
Physical activity: questionnaire		X			X				X					X
NNS intake questionnaire		X	X	X	X	X	X	X	X	X	X	X	X	X
Stress questionnaire		X			X				X					X
Sleep questionnaire		X			X				X					X
Depression questionnaire		X			X				X					X

Antibiotic use		X			X				X					X
Bioelectrical impedance		X			X				X					X
Stool sample collection		X												
Blood sample collection		X			X			X	X					X
Spot urine collection		X			X				X					X
75 g oral glucose tolerance test (OGTT)					X									
TIMEPOINT	***Pre-screen***	**12-18 weeks**	***20 weeks ± 1 week***	***24 weeks ± 1 week***	***28 weeks ± 1 week***	***32 weeks ± 1 week***	***36 weeks ± 1 week***	***Delivery***	***1 mo. PP ± 1 week***	***2 mo. PP ± 1 week***	***3 mo. PP ± 1 week***	***4 mo. PP ± 1 week***	***5 mo. PP ± 1 week***	***6 mo. PP ± 1 week***
		Infant Assessments												
24-hour diet recall in duplicate									X					X
Infant feeding log/weighted feeds									X					X
Body composition (PEA POD)									X					X
Anthropometry														
Stool collection									X					X
Infant feeding questionnaire									X					X

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SPIRIT2025checklistSweetPea9.23.26.docx