Statistical Analysis Plan

Title of the study:	Consumption of Oral Artificial Sweeteners on Platelet Aggregation and Polyol Excretion (COSETTE)
Department	Cardiovascular Medicine, Cleveland Clinic
IRB Number	21-005
Trial registration:	Clinicaltrials.gov ID NCT04731363
Investigators:	
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Original Creation Date	12/21/2020
Version Date	03/25/2022
Activity Log	Details are outlined in the Addendum below under section "History of additional changes of the study protocol or related documents"

Study Overview

The overall purpose of the study COSETTE (Consumption of Oral Artificial Sweeteners on Platelet Aggregation and Polyol Excretion) is to develop a clinical protocol for performance of pilot studies aimed at exploring both changes in blood levels observed following ingestion of a polyol, and its potential impact on platelet function. The initial studies will focus on two polyols that are currently used as artificial sweeteners – erythritol, and xylitol. Studies will proceed focusing individually on each compound sequentially, with studies on each compound being independent of the other. Subject enrolment will proceed for one polyol at a time. COSETTE will thus serve as a template that is to be applied to one compound at a time.

Background:

Erythritol and xylitol are both polyols (compounds with 2 or more hydroxyl groups) found in fruits and vegetables in low amounts. They are widely used artificial sweeteners by the food industry. Due to a sweetening intensity comparable to that of sucrose but lower calorie content erythritol and xylitol are added in large quantities into processed food. Since they do not raise insulin levels and have little to no effect on blood glucose levels, both polyols are also attractive for the use as sugar alternatives in patients with cardiometabolic diseases, such as type 2 diabetes.
Both mentioned sweeteners are generally considered safe and approved by the Food and Drug Administration (FDA). Major side effects of excessive consumption comprise flatulence, laxation, and headache. Erythritol and xylitol are well tolerated up to an intake of 60g and 50g daily, respectively
Up to now, there is no prospective data available about polyols with respect to their impact on event outcomes in cardiovascular patients, despite the extensive use in the food industry. Moreover, little is known about plasma levels and metabolic changes following food intake of artificial sweeteners, in particular polyols.
We have measured fasting levels of various polyols in a large clinical cohort of cardiovascular patients and found that some candidate polyols are related to a higher risk of cardiovascular complications and death. *In vitro* data using human platelets revealed that the polyols xylitol and erythritol at the levels observed in fasting patients enhance platelet aggregation potential. Our data shows that erythritol and xylitol, impact platelet function and thereby may contribute to cardiovascular mortality.

This Statistical analysis plan is based upon the following study documents:

Study Protocol, Version 2.0 (06/07/2021)

Study Objective

The principal goal for the study is to examine whether ingestion of a beverage containing artificial sweeteners alters *in vitro* platelet function. Post prandial levels of polyols following ingestion will also be assessed. We will focus on two popular artificial sweeteners: erythritol and xylitol.

Study Aims

1. To assess post prandial levels of erythritol and xylitol following ingestion of 30g dissolved in water
2. To assess indices of platelet function following erythritol and xylitol ingestion

Study Hypothesis

1. We hypothesize that polyol concentrations in the blood (erythritol and xylitol) increase following ingestion.
2. We hypothesize that post prandial levels of polyols (erythritol and xylitol) alter platelet function.

Study Design

We will perform a prospective, non-blinded, 2-arm, parallel design study with subjects allocated to receive either erythritol or xylitol as their intervention.

At baseline, subjects will have blood and urine samples collected. Subjects will then receive a drink (300mL of water containing up to 30g of erythritol or 30g of xylitol) as a single oral dose. We will use the Now Foods brand of each sweetener and plan to obtain both sweeteners from a commercial source (e.g. Amazon.com or a local pharmacy). Blood and urine samples will be collected again 30 minutes after ingestion (and at time points indicated in the tables below) of the sweetened water drink.

Xylitol arm schedule:

	Visit 1 Day 1	Visit 2 Day 2
Informed Consent	X	
Brief Medical History Questionnaire	X	
Vital Signs	X	
Baseline Fasting Blood Collection	X	X
Baseline Fasting Urine Sample	X	X
Drink Sweetened Water	X	
30 minutes Post-drink Blood & Urine Collection*	X	
1 hour Post-drink Blood & Urine Collection*	X	
4 hours Post-drink Blood & Urine Collection*	X	
6 hours Post-drink Blood & Urine Collection*	X	

Erythritol arm schedule:

	Vital Signs	Medical History	Blood Draw	Spot Urine Sample
Baseline	X	X	X	X
+ 30 minutes			X	X
+ 2 hours*			X	X
+ 6 hours*			X	X
+ 24 hours (Day 2)*			X	X
+ 48 hours (Day 3)*			X	X
+ 72 hours (Day 4)*			X	X
+ 96 hours (Day 5)*			X	X
+ 168 hours (Day 8)*			X	X
+ 216 hours (Day 10)*			X	X

+ 264 hours (Day 12)*			X	X

**Laboratory schedules are subject to change as data is gathered. Subjects will be informed of lab schedule at time of consent.*

Subject Compliance Monitoring

Subjects will be observed taking their assigned intervention, either erythritol or xylitol solution, by a study coordinator or other study personnel during their visit to ensure the subjects are able to comply with the study procedures.

Study Population

There will be 2 different cohorts

Cohort 1 Inclusion Criteria

- Men and women age 18 years or above.
- Able to provide informed consent and comply with study protocol.

Cohort 1 Exclusion Criteria

- Use of anti-platelet medications within 14 days of study enrollment.
- Active infection or received antibiotics within 1 month of study enrollment.
- Use of OTC probiotic within 1 month of study enrollment.
- Diabetes Mellitus
- Ulcerative colitis, Crohn's disease, or other chronic gastrointestinal disorder.
- Past history of bariatric procedures or surgeries (e.g. gastric banding or bypass).
- Pregnancy.
- Significant chronic illness.

- Any condition that, in the judgment of the Investigator, would place a subject at undue risk by being enrolled in the trial or cause inability to comply with the trial.

Cohort 2 Inclusion Criteria

- Men and women age 18 years or above.
- Able to provide informed consent and comply with study protocol.
- Diabetes Mellitus Type II

Cohort 2 Exclusion Criteria

- Use of anti-platelet medications within 14 days of study enrollment.
- Active infection or received antibiotics within 1 month of study enrolment.
- Use of OTC probiotic within 1 month of study enrolment.
- Ulcerative colitis, Crohn's disease, or other chronic gastrointestinal disorder.
- Past history of bariatric procedures or surgeries (e.g. gastric banding or bypass).
- Pregnancy.
- Any condition that, in the judgment of the Investigator, would place a subject at undue risk by being enrolled in the trial or cause inability to comply with the trial.

Subject Recruitment and Screening

Subjects will be recruited from the Cleveland, OH area. Subjects will be recruited by several methods, including (but not limited to) posted advertisements, chart review, and personal interactions with patients in the cardiology outpatient departments at the Cleveland Clinic Foundation. All advertising materials used for this study will be approved by the Cleveland Clinic IRB prior to dissemination.

Subject Consent

Written consent will be obtained from all subjects prior to any study procedure being performed. Participants will be provided with a copy of the signed consent form. Consent will also be documented in the subject's medical record in EPIC.

Early Withdrawal of Subjects

Subjects may withdraw from the trial at any time and for any reason. Some possible reasons for early withdrawal include the following:

- Development of a medical condition or need for concomitant treatment that precludes further participation in the trial
- Removal from the trial by the investigator in the best interests of the subject
- Development of an active infection or starting an antibiotic treatment regimen
- Study completion or discontinuation prior to participant completion
- Subject withdraws consent to continued participation in the trial

Recording of Adverse Events

At each contact with the subject, the investigator will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events will be recorded immediately in the source document. All clearly related signs, symptoms, and abnormal diagnostic procedures results will also be recorded in the source document.

All adverse events will be immediately reported to the primary investigators and to the IRB, per their procedures. The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period will be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

Serious/Unexpected Adverse Events

Serious and unexpected adverse events will be reported to the Ethical Committee in accordance with institutional guidelines. In the case there would be any adverse events or unanticipated problems, an appropriate adverse event or unanticipated report form will be made and the Ethical Committee notified immediately. The study will also be stopped immediately if a subject would experience any unanticipated discomfort or adverse cardiac events (e.g. hospitalization). There will be no DMC and no interim analyses.

Contact information

Contact information for team member responsible for data collection/acquisition:

Jennifer Wilcox, BA, Tel. 216-636-6153, email: kirsopj@ccf.org
Timothy Engelman, LPN, Tel. 216-636-6153, email: engelmt@ccf.org

Data Management

Study data will be collected and managed using REDCap (Research Electronic Data Capture). REDCap is a secure, web application designed to support data capture for research studies, providing user-friendly web-based case report forms, real-time data entry validation (e.g. for data types and range checks), audit trails and a de-identified data export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). The system was developed by a multi-institutional consortium which includes Cleveland Clinic and was initiated at Vanderbilt University. The database is hosted at the Cleveland Clinic Datacenter. The system is protected behind a login and Secure Sockets Layer (SSL) encryption. There is an audit trail tracking all logins and activities in the database. Data collection is customized for each study or clinical trial based on a study-specific data dictionary defined by the research team with guidance from the REDCap administrator in Quantitative Health Sciences at the Cleveland Clinic.

Outcomes

Primary Outcomes

- Polyol levels (erythritol and xylitol) in the blood quantified using established techniques by mass spectrometry.
- Polyol levels (erythritol and xylitol) in the urine quantified using established techniques by mass spectrometry.
- changes in platelet function throughout the study using well established in vitro platelet assays

Secondary Outcomes

- Changes in markers of metabolism

Sample Size Determination

Based on previous research, we calculate that a sample size of 10 will provide us with a power of 0.9 to detect a significant difference between groups at a significance level of 0.05. However, we plan to enrol at least 15 subjects in each cohort in each arm in order to compensate for any unexpected occurrences or subject dropouts.

Demographic and Clinical Characteristics

We plan to use standard descriptive statistics to characterize the overall study population of interest both at baseline and during follow-up. Summary statistics of demographic variables (e.g. age, self-reported race and ethnicity), such as means, medians, standard deviations, and ranges will be produced for measured variables, and compared within and between groups with chi-square, Student t-test/Wilcoxon test, or ANOVA analysis as appropriate.

Statistical analysis Plan for Primary outcomes

1. Erythritol concentration in the blood at various time points will be analyzed by ANOVA analysis or Student t-test/Wilcoxon test as appropriate. Type I error will be controlled for though adjustment for multiple testing as appropriate. A P value <0.05 will be considered significant.

2. Erythritol concentration in the urine at various time points will be normalized to urine creatinine levels: erythritol urine/ creatinine urine. Levels will be analyzed by ANOVA analysis or Student t-test/Wilcoxon test as appropriate. Type I error will be controlled for though adjustment for multiple testing as appropriate. A P value <0.05 will be considered significant.

3. Platelet functional changes before and after xylitol/erythritol drink will be analyzed by ANOVA analysis or Student t-test/Wilcoxon test as appropriate. Type I error will be controlled for though adjustment for multiple testing as appropriate. A P value <0.05 will be considered significant.

Statistical analysis Plan for Secondary outcomes

1. Changes in metabolic markers at various time points will be by ANOVA analysis or Student t-test/Wilcoxon test as appropriate. Type I error will be controlled for though adjustment for multiple testing as appropriate. A P value <0.05 will be considered significant.

Hypothesis testing

For aim 1, we will test the hypothesis that post prandial levels of erythritol or xylitol (after 30g ingestion) exceed fasting levels. Testing will be performed by comparing post prandial erythritol levels with fasting levels in each individual.

For aim 2, we will test the hypothesis that post prandial levels of erythritol and xylitol alter platelet function. Testing will be performed by comparing markers for platelet function before and after exposure to xylitol or erythritol.

Graphical illustration and software used

Graphical methods will be used extensively to examine distributions, identify potential influential points, and guide in data transformations if warranted. Data analysis will be performed with R Software (version 4.0.2, package used for visualization: ggplot2) and GraphPad Prism software (version 9.1.2).

Signatures

Marco Witkowski, MD

W. H. Wilson Tang, MD

Xinmin S. Li, phD

Addendum

History of additional changes of the study protocol or related documents

Protocol changes to version date 06/07/2021:

- Removal of stool sample collection.
- Sample collection schedule has been modified to add more time points for blood collection.
- Subjects will no longer be randomized to their study treatment (polyol). Separate consents will be used for each polyol.
    - We no longer wish to randomize subjects in a 1:1 fashion because equal numbers of subjects in each arm may not be necessary to complete the planned studies. Additionally, subjects consuming xylitol will have a larger amount of blood drawn.
- We are adding a second cohort of subjects with diabetes mellitus to compare the effects of polyols in diabetics versus non-diabetics.

Consent form for Erythritol arm changes to version date 03/25/2022:

- Increase in the maximum blood draw volume for Day 1.