Consumption of Oral Artificial Sweeteners on Platelet Aggregation and Polyol Excretion (COSETTE)

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1.0 BACKGROUND AND SIGNIFICANCE

The principal goals for the study are to examine 1) how long after ingestion of a single sweetened beverage are levels of that sweetener still elevated, and 2) whether ingestion of a beverage containing either an artificial sweetener or glucose alters *in vitro* platelet aggregation.

Due to the growing number of cardiometabolic diseases like diabetes mellitus, the use of polyols (a.k.a. sugar alcohols) and other artificial sweeteners to replace free sugars has recently gained increasing appreciation. Two popular artificial sweeteners are erythritol and xylitol.

Erythritol and xylitol are both naturally occurring polyols found in fruits and vegetables. They are potent artificial sweeteners with a higher sweetening intensity and lower calorie content than table sugar. This makes them attractive for the use as sugar substitutes or alternatives, particularly for patients with type 2 diabetes.

All three mentioned sweeteners are generally considered safe and approved by the Food and Drug Administration (FDA) as food additives. 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 (1, 2). Major side effects of glucose are insulin resistance and type 2 diabetes following excessive chronic consumption.

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

induce platelet aggregation potential. Our data shows that erythritol and xylitol, impact platelet function and thereby may contribute to cardiovascular mortality.

In preliminary studies we found that upon ingestion erythritol and xylitol levels in the plasma rise within the first hour. With this study we wish to examine whether the postprandial levels are capable of altering platelet function *in vitro*. We hypothesize that post prandial polyol concentrations following ingestion increase platelet aggregation in the blood.

Glucose, as a naturally occurring sweetener, will serve as a control for this study. There is also evidence that glucose may increase platelet aggregation, allowing us to compare the degree of increased aggregation between the polyol sweeteners and glucose. (3)

2.0 STUDY OBJECTIVES

To assess platelet function before and after polyol exposure.

3.0 STUDY DESIGN

We will perform a prospective, non-blinded, 4-arm study. There will be up to 8 study visits depending on which arm the subject is assigned to. There will be three interventions: erythritol, xylitol, and glucose. Erythritol and glucose will each be used in one arm, xylitol will be used in two arms.

At baseline, subjects will have fasting blood and urine samples collected. Subjects will then receive a drink (300mL of water containing either 30g of erythritol, 30g of xylitol, 5g of xylitol, or 30g of glucose) as a single oral dose. Blood and urine samples will be collected again on the same day at pre-determined intervals after ingestion of the sweetened water drink. Additional fasting blood and urine samples may be collected on subsequent days after drinking the artificially sweetened water as shown in the tables below. Subjects drinking xylitol (5g or 30g) may have 1 subsequent visit. Subjects consuming erythritol may have up to 7 subsequent visits. Subjects consuming glucose will have 1 study visit with blood and urine being collected about 30 minutes apart. All fasting collections will occur after an 8+ hour fast.

Xylitol schedule (5g and 30g):

	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 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

Glucose schedule:

	Vital Signs	**Medical History**	**Blood Draw**	**Spot Urine Sample**
Baseline	X	X	X	X
+ 30 minutes			X	X

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

3.1 Study endpoints

- Baseline plasma and urine fasting polyol or glucose

- Baseline and subsequent (after sweetener intake) changes in levels of polyol or glucose in plasma and urine as measured by established techniques by mass spectrometry.
- Baseline and subsequent (after sweetener intake) changes in platelet function throughout the study using well established in vitro platelet assays.

- Changes in markers of metabolism (e.g. lipid profile).

4.0 SUBJECT SELECTION AND WITHDRAWAL

There will be two study cohorts for the polyol arms. We will enroll healthy control subjects in the glucose arm. Subjects must meet the following inclusion/exclusion criteria to be eligible for the study.

4.1 Cohort 1 Inclusion Criteria

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

4.2 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.

4.3 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

4.4 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 enrollment.
- Use of OTC probiotic within 1 month of study enrollment.
- 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.

4.5 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.

4.6 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.

4.7 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

5.0 STUDY DRUG/FOOD ADDITIVE

5.1 Description

Erythritol and xylitol supplements: The brand of supplements to be used is NOW Foods. These will be obtained from a commercial source, either Amazon.com or a pharmacy/store carrying this brand and stored as below.
Glucose: The brand of glucose is NOW Foods. We will obtain from Amazon.com or a pharmacy/store carrying this brand. It will be stored at room temperature in a sealed bag.

5.2 Treatment Regimen

The study additive – erythritol, xylitol, or glucose – will be dissolved in 300mL of water and given to the subjects as a water solution to be taken all at once by mouth. The 300mL of water will contain either 30g of erythritol, 30g of xylitol, 5g of xylitol, or 30g of glucose depending on the intervention the subject is assigned to.

5.3 Subject Compliance Monitoring

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

5.4 Storage

Erythritol, xylitol and glucose supplements will be stored at room temperature in a dry location. They will be kept locked in a secure location. Temperature will be monitored and recorded weekly to make sure that the temperature remains between 15° and 30° Celsius.

6.0 STUDY PROCEDURES

This is a prospective pilot study. Up to 105 subjects will be enrolled in this pilot study, with a goal of having at least 12 subjects in each cohort of the polyol sweetener arms & 12 subjects in the glucose arm complete the study.

6.1 Study Visit Procedures

- Blood Draws
- Spot Urine collections
- Medical History
- Medication Review
- Physical Exam including blood pressure, height, weight, and waist circumference

7.0 STATISTICAL PLAN

7.1 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 enroll at least 15 subjects in each cohort in each arm in order to compensate for any unexpected occurrences or subject dropouts.

7.2 Statistical Methods

We plan to use standard descriptive statistics to characterize the overall study population of interest both at baseline and during follow-up. Summary statistics 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. Graphical methods will be used extensively to examine distributions, identify potential influential points, and guide in data transformations if warranted.

8.0 SAFETY AND ADVERSE EVENTS

8.1 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.

8.2 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.

9.0 DATA HANDLING AND RECORD KEEPING

9.1 Confidentiality and Privacy

Information about study subjects will be kept confidential and managed according to the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Subjects will be informed of their rights and the privacy safeguards in place during the consent process.

9.2 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.

9.3 Records Retention

All source documents, including the case report forms, will be kept for at least 6 years following the completion of the study. Documents will be stored in a secure location with limited access.

10.0 STUDY MONITORING, AUDITING AND INSPECTING

10.1 Study Monitoring

The Investigator will ensure that any monitor or other compliance or quality assurance reviewer is given access to all study-related documents and study related facilities, and that they have adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and Institutional compliance and quality assurance groups of all study related documents (for example, source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (for example, pharmacy, diagnostic laboratory, etc.). Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable Institutional compliance and quality assurance offices.

11.0 ETHICAL CONSIDERATIONS

This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB

concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

12.0 REFERNCES

1. Mäkinen KK. Gastrointestinal Disturbances Associated with the Consumption of Sugar Alcohols with Special Consideration of Xylitol: Scientific Review and Instructions for Dentists and Other Health-Care Professionals. Int J Dent. 2016;2016:5967907-.
2. Oku T, Okazaki M. Laxative threshold of sugar alcohol erythritol in human subjects. Nutrition Research. 1996;16(4):577-89.
3. Sudic D, Razmara M, Forslund M, Ji Q, Hjemdahl P, Li N. High glucose levels enhance platelet activation: involvement of multiple mechanisms. Br J Haematol. 2006 May;133(3):315-22. doi: 10.1111/j.1365-2141.2006.06012.x. PMID: 16643434.