Effect of the natural sweeteners erythritol and xylitol on gut microbiota and glucose metabolism in obese volunteers: a pilot study

Clinical Study Protocol

A single centre; randomized parallel-group trial.

Study Type:	Clinical trial with life-style intervention (nutrition)
Study Categorisation:	A according to ordinance HRO Art.7
Study Registration:	Name of the intended registry: Clinical Trial Gov
Study Identifier:	None
Sponsor:	St. Claraspital, Abteilung für Forschung
Principal Investigator:	Name: *Dr. Bettina Wölnerhanssen* Address: *St. Claraspital, Abteilung für Forschung* *Kleinriehenstrasse 30, 4016 Basel* Email: *bettina.woelnerhanssen@claraspital.ch* Phone: *+41 61 685 86 32*
Investigational Product:	Xylitol (E967) and Erythritol (E968)
Protocol Version and Date:	Version 2, 6.6.2016, Version 3, 25.9.2017

Signature Page(s)

Study number	EKNZ BASEC 2016-00782
Study Title	Effect of the natural sweeteners erythritol and xylitol on gut microbiota and glucose metabolism in obese volunteers: a pilot study

The Sponsor-Investigator and trial statistician have approved the protocol version 3, 25.9.2017 and confirm hereby to conduct the study according to the protocol, current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm if applicable and the local legally applicable requirements.

Sponsor-Investigator: Dr. Bettina Wölnerhanssen

Basel, 25.9.2017 — Dr. med. Bettina Wölnerhanssen, Oberärztin, Forschung und Dokumentation, St. Claraspital, 4016 Basel

Place/Date | Signature

Local Principal Investigator at study site*:

I have read and understood this trial protocol and agree to conduct the trial as set out in this study protocol, the current version of the World Medical Association Declaration of Helsinki, ICH-GCP guidelines or ISO 14155 norm and the local legally applicable requirements.

Site	St. Claraspital Basel, Abteilung für klinische Forschung, Kleinriehenstrasse 43, 4016 Basel
Principal investigator	Dr. Bettina Wölnerhanssen

Dr. med. Bettina Wölnerhanssen, Oberärztin, Forschung und Dokumentation, St. Claraspital, 4016 Basel

Basel, 25.9.2017

Place/Date | Signature

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STUDY SYNOPSIS

Sponsor **Principal Investigator**	Abteilung für Forschung St. Claraspital Basel Dr. Bettina Wölnerhanssen Abteilung für Forschung St. Claraspital Basel Kleinriehenstrasse 30 4016 Basel Phone: 0041 61 685 86 32 Email: bettina.woelnerhanssen@claraspital.ch
Study Title:	Effect of the natural sweeteners erythritol and xylitol on gut microbiota and glucose tolerance in obese volunteers: A pilot study
Short Title / Study ID:	n/a
Protocol Version and Date:	Version 2, 6.6.2016, Version 3, 25.9.2017
Trial registration:	Clinical Trial Gov
Study category and Rationale	A: Study without drugs, without medical devices with low risk: life-style intervention study using food additives (E967 and E968).
Background and Rationale:	Sugar alcohols such as xylitol and erythritol are increasingly popular as sugar substitutes in the food industry and are also recommended to diabetic patients. Both substances are already in use in the food industry and are freely available (e.g. in health food or drug stores). Xylitol (E967) is a sweet substance naturally found in low concentrations in vegetables (such as cauliflower) and fruit (plums, strawberries, and raspberries). Xylitol is as sweet as table sugar, but has around 40% less calories than ordinary sugar. After an intake of xylitol, only a weak rise in blood sugar and insulin levels can be observed. Erythritol (E968) is also a sugar alcohol and is naturally found in mushrooms and soy. Erythritol is less sweet than table sugar (approximately 70% of the sweetness of table sugar), has no calories at all and has no effect on blood sugar or insulin levels. Since the 1970s, beneficial effects on oral health could be demonstrated as oral bacteria were influenced positively (caries reduction, Makinen 2011). Interest now arises because of the multiple other potential health benefits that have been observed in studies examining the two substances. Especially the sugar metabolism seems to be influenced positively. Sugar (glucose) metabolism is often impaired in the obese, even in the absence of diabetes mellitus. In animal studies, xylitol consumption stimulated the insulin-producing islet cells in the pancreas (Rahman 2014), led to a reduction in abdominal fat (Amo 2011) and improved sugar metabolism (Kishore 2012). In the 1980ies animal studies showed an increase in gut Clostridium perfringens after xylitol intake; certainly a non-desirable effect1. However, studies on effects of erythritol and xylitol on the human gut microbiota are lacking so far. A global metabolomic analysis represents a most-promising approach to comprehensively characterize metabolic changes associated with obesity and dietary interventions, as it can capture a wide range of metabolic processes at the same time. In this trial, we aim to examine whether gut microbiota and glucose tolerance can be influenced by polyol intake in a non-diabetic but obese cohort.
Objective(s):	Effect of daily intake of xylitol or erythritol over 7 weeks on gut microbiota and glucose tolerance

Outcome(s):	Primary endpoint: 1) Glycaemic control (fasting insulin & glucose & c-peptide after oral glucose challenge) Secondary endpoints: 2) Human gut microbiota composition 3) Metabolomics 4) Blood lipid profile and hepatic enzymes 5) Inflammatory response 6) Intestinal permeability 7) Gastrointestinal tolerance (bloating, diarrhoea etc.) 8) intestinal glucose absorption
Study design:	This pilot trial is a randomized, controlled interventional study; the project set-up will be single-centre, national.
Inclusion / Exclusion criteria:	Inclusion criteria: - Obese volunteers (BMI > 30kg/m2) - Aged 18- max. 55 years - Otherwise healthy. Exclusion criteria: - Known cardiovascular disease - Diabetes mellitus treated by oral antidiabetics or insulin - Arterial hypertension with antihypertensive treatment - Dyslipidaemia with statin therapy - Known chronic hepatic disease (NASH, hepatitis). - Known renal disease: kidney failure - Pregnancy - Intake of proton pump inhibitors (PPIs) on a regular basis - Intake of antibiotics within the last 3 months before inclusion - Intake of pro or prebiotics - Chronical diseases of the gastrointestinal tract, history of gastrointestinal surgery with major changes to the gastrointestinal tract - Substance abuse, alcohol abuse. - Inability to follow procedures due to psychological disorders, dementia or insufficient knowledge of project language (German).
Measurements and procedures:	Over a period of 7 weeks, volunteers will ingest the natural sweetener xylitol or erythritol (or no additional sweetener). Once before and after these 7 weeks we will carry out an oral glucose tolerance test, examine faeces and urine (gut microbiota and metabolomics) and take blood samples to analyse lipid profile and liver enzymes. Volunteers will fill in a food diary and a questionnaire for gastrointestinal symptoms. The whole study will take approximately 8 weeks.
Study Product / Intervention:	Randomization to the three study arms will be done by a computer based randomization (excel). The control group will not receive any sweetener. The two intervention groups will receive either xylitol or erythritol as follows: 1st week = run-in (adaption week) - 2 days 1 *portion per day (1-0-0) - 3 days 2 portions per day (1-1-0) - 2 days 3 portions /day (1-1-1) 2nd to 7th week: Over four weeks the volunteers will ingest 3 portions/day. *1 portion xylitol = 8g, 1 portion erythritol = 12g
Control Intervention	We will compare a) Baseline findings to post treatment findings. In this way each volunteer will serve as his/her own control. b) A control group will show whether findings are stable within a participant over 7 weeks.
Number of Participants with Rationale:	In total will enrol 60 volunteers, of which 20 will be controls, 20 will be treated with xylitol and 20 erythritol. This is a pilot trial and a power analysis is not possible. However, we assume that a relevant difference between the treatments should be observed in 20 subjects in each group.
Study Duration:	We plan to carry out this trial within approximately 1 year, starting in August 2016 till October 2018, depending on recruitment.
Study Schedule:	Month Year of First-Participant-In (planned): 1st August, 2016 Month Year of Last-Participant-Out (planned): 1st October, 2018
Investigator(s):	Dr. Bettina Wölnerhanssen Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel Email: Bettina.Woelnerhanssen@claraspital.ch Phone: +41 61 685 86 32

Study Centre(s):	Single-centre
Statistical Considerations:	We will compare a) baseline findings to post treatment findings. In this way each volunteer will serve as his/her own control. In addition, b) a control group will show whether findings are stable within a participant over 7 weeks. This is a pilot trial and a power analysis is not possible. However, we assume that a relevant difference between the treatments should be observed in 20 subjects in each group.
GCP Statement:	This study will be conducted in compliance with the protocol, the current version of the Declaration of Helsinki, the ICH-GCP as well as all national legal and regulatory requirements.
Risk-Benefit statement:	The excessive consumption of erythritol and xylitol can lead to bloating or diarrhoea in sensitive patients. The dosage at which these problems occur varies from person to person, but in most cases single dosage has to exceed 20g per portion or exceed 50g per day. The given dosage in the study will be spread out over the day and we do not anticipate any problems. In previous research, the body was shown to have acclimatised itself to the substances within a few days. The participants are subject to only minimal risk during this study, such as through the blood collection. At the puncture site, swelling, reddening or hematoma can occur, which usually resolves after a couple of days. In rare cases, a local infection or local thrombophlebitis can occur at the puncture site.

STUDY SUMMARY IN LOCAL LANGUAGE

Sponsor **Principal Investigator**	Abteilung für Forschung, St. Claraspital Basel Dr. Bettina Wölnerhanssen Abteilung für Forschung St. Claraspital Basel Kleinriehenstrasse 30 4016 Basel Phone: 0041 61 685 86 32 Email: bettina.woelnerhanssen@claraspital.ch
Study Title:	Effekt von den natürlichen Süssungsmitteln Erythrit und Xylit auf die Darmbakterien und den Zuckerstoffwechsel bei übergewichtigen Personen: eine Pilotstudie
Short Title / Study ID:	-
Protocol Version and Date:	Version 2, 6.6.2016, Version 3 25.9.2017
Trial registration:	Clinical Trial Gov
Study category:	Risiko Kategorie A gemäss HRO Art.7
Type of Research:	Eine klinische Studie ohne Medikamente oder medizinische Geräte mit niedrigem Risiko unter Verwendung von Nahrungsmittelzusätzen (E967 und E968), bei welchem biologisches Material und gesundheitsbezogene Daten verschlüsselt gesammelt werden.
Background and Rationale:	Bei krankhaft Übergewichtigen (Body Mass Index über 35kg/m2) kommt es zu zahlreichen Folgeerkrankungen wie metabolem Syndrom, Bluthochdruck und Vorstufen von Diabetes. Xylit und Erythrit werden seit den 1970er Jahren vor allem in der Zahnhygiene eingesetzt, da sich gezeigt hat, dass diese Substanzen die Zusammensetzung der Speichelbakterien günstig beeinflusst (Kariesreduktion). Xylit scheint zudem auch positive Effekte auf den Stoffwechsel zu haben, insbesondere auf die Zuckerverwertung, die bei Übergewichtigen oft gestört ist auch wenn keine Zuckerkrankheit vorliegt. Im Tierversuch zeigt sich, dass Xylit die insulinproduzierenden Inselzellen in der Bauchspeicheldrüse anregt, zu einer Reduktion des Bauchfettes führt und die Zuckerverwertung verbessert. Bei künstlichen Süssungsmitteln wie Aspartam und Acesulfam-K konnte in einer Studie gezeigt werden, dass die Darmbakterienzusammensetzung sich verändert und es zu einer Störung der Zuckerverwertung kommt. Über die Auswirkung von Xylit und Erythrit auf die Darmbakterien ist bisher noch nichts bekannt. Mit dieser Studie möchten wir diese Effekte bei einem nicht-diabetischen, aber übergewichtigen Kollektiv (BMI ab 30kg/m2) prüfen.

Objective(s):	Wir möchten untersuchen, ob Erythrit- und Xyliteinnahme als Süssungsmittel einen Effekt auf die Darmbakterienzusammensetzung und die Zuckerverwertung hat
Outcome(s):	Primärer Endpunkt: - Effekte auf die Zuckerverwertung (oraler Glukosetoleranztest) Sekundäre Endpunkte: - Effekte auf die Zusammensetzung der Darmbakterien - Effekte auf den Stoffwechsel (Metabolomics) - Effekte auf die Darmpermeabilität - Effekte auf den Entzündungszustand - Effekte auf die intestinale Glukoseabsorption
Study design:	Interventionell-experimentelle, randomisierte, Parallelstudie
Inclusion / Exclusion criteria:	In diese Studie sollen insgesamt 60 adipöse Probanden (BMI ≥ 30kg/m2), Frauen und Männer (Alter: 18-55 Jahre), eingeschlossen werden. Allgemein gute körperliche Verfassung, kein Diabetes mellitus, keine arterielle Hypertonie, keine vorgängige Operation wie Magenbypass, keine Erkrankungen des Magendarmtraktes. Keine Antibiotikatherapie in den letzten 3 Monaten vor Studienbeginn.
Measurements and procedures:	Die Probanden werden über sieben Wochen entweder 24g Xylitol oder 36g Erythritol oder aber nichts Zusätzliches (Kontrollgruppe) einnehmen. Vor und nach dieser Periode wird ein oraler Glukosetoleranztest, eine Blutprobe, eine Stuhl und Urinprobe abgenommen. Weiter wird ein Ernährungstagebuch geführt und Symptome des Magendarmtraktes festgehalten..
Study Product / Intervention:	Eine Randomisierung zu den drei Testarmen wird mit einem computer-basierten Zufallsgenerator vorgenommen (Excel). Die Kontrollgruppe erhält kein Süssungsmittel. Die zwei Interventionsgruppen bekommen folgendes Verabreichungsschema: Erste Woche = “run-in” (Adaptionswoche) - 2 Tage 1 *Portion pro Tag (1-0-0) - 3 Tage 2 Portionen pro Tag (1-1-0) - 2 Tage 3 Portionen pro Tag (1-1-1) 2-7. Woche: Während vier Wochen werden jeweils 3 Portionen/Tag eingenommen. *1 Portion Xylitol = 8g, 1 Portion Erythritol = 12g
Control Intervention	Wir vergleichen: a) die Ausgangswerte zu den Werten nach der Behandlung. b) Eine Kontrollgruppe (ohne Behandlung) wird zeigen, ob die Befunde bei zweimaliger Erhebung innert 7 Wochen stabil sind.
Number of Participants with Rationale:	Insgesamt sollen 60 Probanden eingeschlossen werden, wobei 20 zur Erythrit, 20 zur Xylit und 20 zur Kontrollgruppe (ohne Süssungsmittel) randomisiert werden. Eine Poweranalyse ist bei der aktuellen Datenlage nicht möglich. Wir gehen aber davon aus, dass diese Anzahl Teilnehmende ausreichen sollte um einen relevanten Unterschied zu finden.
Study Duration/Schedule:	Studienbeginn geplant: August 2016, Studienende: Oktober 2018
Study Centre(s):	Diese Studie wird an einer Institution durchgeführt (St. Claraspital Basel)
Statistical Considerations:	Da es sich um eine Pilotstudie handelt und kaum Daten zur Verfügung stehen, ist eine Poweranalyse zum jetzigen Zeitpunkt nicht möglich. Wir gehen aber davon aus, dass relevante Veränderungen bei mind. 20 Probanden in jeder Gruppe statistische Signifikanz erreichen sollten
GCP Statement:	Diese Studie wird gemäss der Deklaration von Helsinki, ICH-GCP und gemäss Schweizer Recht durchgeführt
Risk-Benefit statement:	Erythrit und Xylit werden in der Lebensmittelindustrie eingesetzt und können bei empfindlichen Personen bei übermässigem Verzehr zu Diarrhoe und Blähungen führen. Die gewählten Dosen sollten, wenn sie wie geplant über den Tag verteilt eingenommen werden und eine Angewöhnungszeit angeboten wird, keine Nebenwirkungen haben. Sollte sich zeigen, dass sich Erythrit und Xylit positiv auf die Glukosetoleranz auswirken, könnten in Zukunft übergewichtige und diabetische Patienten von dieser Süssungsalternative profitieren.

ABBREVIATIONS

AE	Adverse Event
CEC	Competent Ethics Committee
CRF	Case Report Form
ClinO	Ordinance on Clinical Trials in Human Research *(in German: KlinV, in French: OClin)*
CTCAE	Common terminology criteria for adverse events
DSUR	Development safety update report
GCP	Good Clinical Practice
IB	Investigator's Brochure
Ho	Null hypothesis
H1	Alternative hypothesis
HFG	Humanforschungsgesetz (Law on human research)
HMG	Heilmittelgesetz
HRA	Federal Act on Research involving Human Beings
IMP	Investigational Medicinal Product
IIT	Investigator-initiated Trial
ISO	International Organisation for Standardisation
ITT	Intention to treat
KlinV	Verordnung über klinische Versuche in der Humanforschung *(in English: ClinO, in French OClin)*
LPTh	Loi sur les produits thérapeutiques
LRH	Loi fédérale relative à la recherche sur l'être humain
MD	Medical Device
OClin	Ordonnance sur les essais cliniques dans le cadre de la recherche sur l'être humain *(in German : KlinV, in English : ClinO)*
PI	Principal Investigator
SDV	Source Data Verification
SOP	Standard Operating Procedure
SPC	Summary of product characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

SCHEDULE OF ASSESSMENTS (FLOW OF RESEARCH PROJECT)

Project Periods	**Screening Visit (V1)**	**Baseline Metabolic Visit (V2)**	**Intake of sweetener:** • erythritol or xylitol • week 1: adaption • week 2-7: 3 x/day							**Follow-up Metabolic Visit (V3)**
Location	St. Claraspital	St. Claraspital								St. Claraspital
Time (hour, day, week)	-7d	0d								49d +max. 3 days
			Weeks							
			1	2	3	4	5	6	7	
Participant Information and Informed Consent	x		**Adaption week with increasing doses:** • 2 days 1 portion (1-0-0) • 3 days 2 portions (1-1-0) • 2 days 3 portions (1-1-1)							
Demographics: weight, height, abd. circumference	x									x
Medical History	x									
In- /Exclusion Criteria	x									
Randomization		x								
Fasting blood sample	x	x								x
• Haemoglobin	x									
• Leucocytes	x									x
• HbA1C	x									
• Liver enzymes: ALAT, ASAT	x									x
• Creatinine	x									
• Lipid panel (Triglyc., total Chol., HDL, LDL)	x									x
• hsCRP	x									x
• Serum aliquots for: - IL-6, zonulin - TNF-α - sTNFR - sTNFR2 - LPS		x								x
Urine Pregnancy Test	x									
Oral glucose tolerance test 75g glucose (0-30- 60-90-120min)		x								x
• Blood glucose		x								x
• Insulin		x								x
• C-peptide		x								x
• 3-O-Methyl-D-Glucose		x								x
Urine sample		x								x
Faecal sample		x								x
GSRS: questionnaire gastrointestinal symptoms	x				x				x	
Food diary		x			x				x	
Follow-up calls				x	x	x	x	x	x	
SAE				x	x	x	x	x	x	x
End of study meeting										x

1. STUDY ADMINISTRATIVE STRUCTURE

1.1 Sponsor, Sponsor-Investigator /Principal Investigator(s)

Sponsor	St. Claraspital, Abteilung für Forschung
Project Leader and Coordinating researcher	Name: Dr. Bettina Wölnerhanssen Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel Email: bettina.woelnerhanssen@claraspital.ch Phone: +41 61 685 86 32
Project site(s) and responsible researcher:	Institution: St. Claraspital Basel Name: Dr. Bettina Wölnerhanssen Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel Email: bettina.woelnerhanssen@claraspital.ch Phone: +41 61 685 86 32

1.2 Statistician ("Biostatistician")

Biostatistican	Name: Prof. Dr. Jürgen Drewe Address: Universitätsspital Basel, Petersgraben 4 4031 Basel. +41 61 265 47 35 Email: juergen.drewe@unibas.ch Phone: +41 61 265 47 35

1.3 Laboratory

Not defined yet.

1.4 Monitoring institution

N/A.

1.5 Data Safety Monitoring Committee

N/A.

1.6 Any other relevant Committee, Person, Organisation, Institution

Key Persons involved in research project:	Name: Prof. Christoph Beglinger Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel Email: christoph.beglinger@claraspital.ch Phone: +41 61 685 86 31 Name: Dr. phil. Anne Christin Meyer-Gerspach Function: Post Doc Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel (from 1st July, 2016). Email: annechristin.meyergerspach@kuleuven.be Name: Dorothea Brosi Function: Study nurse Address: St. Claraspital, Abteilung für Forschung Kleinriehenstrasse 30, 4016 Basel Email: dorothea.brosi@claraspital.ch Phone: +41 61 685 86 48
Other personnel	Masterstudent (non-nominatus)
Collaborators:	Name: Prof. Dr. Patrice D. Cani Address: Louvain Drug Research Institute, Metabolism and Nutrition, WELBIO, Walloon Excellence in Life sciences and BIOtechnology, NeuroMicrobiota lab, European Associated

Laboratory (INSERM/UCL), Av. E. Mounier, 73 box B1.73.11, B-1200 Brussels, BELGIUM
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ETHICAL AND REGULATORY ASPECTS

The decision of the CEC concerning the conduct of the study will be made in writing to the Sponsor-Investigator before commencement of this study. The clinical study can only begin once approval from all required authorities has been received. Any additional requirements imposed by the authorities shall be implemented.

1.7 Study registration

This trial is intended to be registered at Clinical Trial Gov. In addition registration in a national language in the Swiss Federal Complementary Database (Portal) will be done.

1.8 Categorisation of study

Erythritol (E968) and xylitol (E967) are food additives used in the food industry (GRAS- generally regarded as safe) and intake involves no risk for the volunteers. The risk category is therefore A (according to HRO Art. 7).

1.9 Competent Ethics Committee (CEC)

Before the project will be conducted, the project plan, the proposed participant information and consent form as well as other project-specific documents shall be submitted to the local Ethics Committee (EC). All changes in research activity and all unanticipated problems involving risks to humans; including the case of planned or premature project end will be reported to the EC. In addition we will provide a final report to the EC within 90 days upon completion of the project (HRO Art. 22).

1.10 Ethical Conduct of the Study

The study will be carried out in accordance to the protocol and with principles enunciated in the current version of the Declaration of Helsinki, the guidelines of Good Clinical Practice (GCP) issued by ICH, the Swiss Law and Swiss regulatory authority's requirements. The CEC and regulatory authorities will receive annual safety and interim reports and be informed about study stop/end in agreement with local requirements.

1.11 Declaration of interest

The principal investigator and all persons involved in this trial declare there is no conflict of interest.

1.12 Patient Information and Informed Consent

The investigators will explain to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits and any discomfort it may entail. Each participant will be informed that the participation in the study is voluntary and that he/she may withdraw from the study at any time and that withdrawal of consent will not affect his/her subsequent medical assistance and treatment. The participant must be informed that his/her medical records may be examined by authorised individuals other than their treating physician. All participants for the study will be provided a participant information sheet and a consent form describing the study and providing sufficient information for participant to make an informed decision about their participation in the study. The participant will be given at least 24 hours to make an informed decision. The patient information sheet and the consent form will be submitted to the CEC and to the competent authority (as applicable) to be reviewed and approved. The formal consent of a participant, using the approved consent form,

must be obtained before the participant is submitted to any study procedure. The participant should read and consider the statement before signing and dating the informed consent form, and should be given a copy of the signed document. The consent form must also be signed and dated by the investigator (or his designee) and it will be retained as part of the study records.

1.13 Participant privacy and confidentiality

The investigator affirms and upholds the principle of the participant's right to privacy and that they shall comply with applicable privacy laws. Especially, anonymity of the participants shall be guaranteed when presenting the data at scientific meetings or publishing them in scientific journals.
Individual subject medical information obtained as a result of this study is considered confidential and disclosure to third parties is prohibited. Subject confidentiality will be further ensured by utilising subject identification code numbers to correspond to treatment data in the computer files.
For data verification purposes, authorised representatives of the Sponsor (-Investigator), a competent authority (e.g. Swissmedic), or an ethics committee may require direct access to parts of the medical records relevant to the study, including participants' medical history.

1.14 Early termination of the study

Compliance is of uttermost importance in this project. The trial might be terminated in case volunteers report insufficient intake of the described substances. Also, in case too many volunteers describe intolerable gastrointestinal side effects, we would have to reconsider the study setup.
The premature end or interruption of the research project is reported to the CEC within 90 days upon completion of the project (HRO Art. 22).
The Sponsor-Investigator may terminate the study prematurely according to certain circumstances, for example:

- ethical concerns,
- insufficient participant recruitment,
- when the safety of the participants is doubtful or at risk, respectively,
- alterations in accepted clinical practice that make the continuation of a clinical trial unwise,
- early evidence of benefit or harm of the experimental intervention

1.15 Protocol amendments

In case significant changes to the project plan (such as changes to eligibility criteria, outcomes, analyses) are necessary, we will send an amendment addressing these questions to the CEC for approval. The Sponsor or Project Leader will in this case submit to the CEC any application documents which are affected by the change. At the same time, the project leader shall provide information on the reasons for the change. Substantial amendments are only implemented after approval of the CEC.
Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the sponsor and the CEC. Such deviations shall be documented and reported to the sponsor and the CEC as soon as possible.
All Non-substantial amendments are communicated to the CEC within the Annual Safety Report (ASR).

2. BACKGROUND AND RATIONALE

2.1 Background and Rationale

Obesity has increased significantly worldwide[1]. In morbid obesity virtually all patients suffer from metabolic syndrome with non-alcoholic fatty liver disease, dyslipidaemia and impaired glycaemic control. Sugar consumption - in the form of sucrose or high-fructose corn syrup (HFCS) - has partly contributed to the dramatic rise in obesity, metabolic syndrome and diabetes[2,3]. Research on the effects of dietary sugars on health has recently focused on fructose, given the striking parallel increases in obesity and in fructose intake over the past decades[4,5]. Fructose intake in diets mostly originates from sucrose (containing 50% fructose and 50% glucose) and soft drinks containing high-fructose corn syrup (HFCS)[6]. Patients with non-alcoholic fatty liver disease (NAFLD) consume double the amount of calories of HFCS from beverages than healthy patients[7]. The increasing evidence of the detrimental role of sucrose and fructose justifies a reduction in intake and substitution of sugar by alternative dietary sweeteners. However, alternatives are not so easy to find.
Various nutrients have shown to influence gut microbiota positively or negatively. Lately, a study was published demonstrating that consumption of commonly used artificial sweeteners drive the

development of glucose intolerance through induction of compositional and functional alterations to the intestinal microbiota[8].

Xylitol and erythritol are sweeteners naturally found in low concentrations in fruit and vegetables, and can be extracted from fibrous material such as birch and used as natural sugar replacers. Since the 1970ies beneficial effects on oral health could be demonstrated as oral microbiota are influenced positively by the reduction of *Streptococcus mutans*, as an example[9]. Interest now arises because of multiple potential health benefits that have been observed in studies examining polyols: apart from being non-cariogenic they are low glycaemic, low-energy and low-insulinaemic, and low-digestible and seem to act as hydroxyl radical scavenger[10]. In rats, xylitol consumption suppressed high-fat induced visceral fat accumulation[11], improved islet cell morphology[12] and led to amelioration of glycaemic control[13]. In a human study carried out on lean subjects in 1982, 3 weeks of xylitol intake showed a decrease in plasma cholesterol values[14]. Results from our own research show deceleration of gastric emptying and a marked increase in GLP-1 and CCK after xylitol and erythritol consumption[15]. Stimulation of incretin release might in part be responsible for amelioration of glycaemic control

In the 1980ies animal studies showed an increase in *Clostridium perfringens* after xylitol intake; certainly a non-desirable effect[16]. However, studies on effects of erythritol and xylitol on the human gut microbiota are lacking so far. A global metabolomic analysis represents a most-promising approach to comprehensively characterize metabolic changes associated with obesity and dietary interventions, as it can capture a wide range of metabolic processes at the same time[17,18]. The metabolome can be considered an integrative downstream product of the genome, transcriptome, proteome, and gut microbiome–host interaction and also behavioural aspects such as dietary habits[19].

Results from animal and human studies suggest a positive effect of erythritol and xylitol on glycaemic control. Alterations in gut microbiota might contribute to such an effect. With this trial we aim to get more insight into the metabolic effects of erythritol and xylitol. We hypothesize that glycaemic control might be ameliorated in obese, non-diabetic (but insulin resistant) patients and gut microbiota are influenced positively.

2.2 Investigational Product

Xylitol (E967) and erythritol (E968) are sweeteners naturally found in low concentrations in fruits and vegetables, and can be extracted from fibrous material such as birch and used as natural sugar replacers. Since the 1970ies beneficial effects on oral health could be demonstrated as oral microbiota is influenced positively with reduction of Streptococcus mutans, as an example[9]. Interest now arises because of multiple potential health benefits that have been observed in studies examining polyols: apart from being non-cariogenic they are low glycaemic, low-energy and low-insulinaemic, and low-digestable and seem to act as hydroxyl radical scavenger[10]. In rats, xylitol consumption suppressed high-fat induced visceral fat accumulation[11], improved islet cell morphology[12] and lead to amelioration of glycaemic control[13]. Results from our own research show deceleration of gastric emptying and a marked increase in GLP-1 and CCK after xylitol and erythritol consumption[15]. Stimulation of incretin release might in part be responsible for amelioration of glycaemic control.

2.3 Dose Rationale

1) Tolerance: Previous trials have been carried out with polyol doses up to 200g per day, however if 50g or more per day are consumed, the risk of gastrointestinal discomfort increases.
2) Previous metabolic effects: A pilot trial by Flint et al. could show metabolic effects after 4 weeks of 36g of erythritol consumption in that vascular function improved[20].
3) Average sugar consumption: Average daily sugar consumption in Switzerland is estimated to be ca. 120g of sucrose/d. Of these 120g an estimated 80% is consumed as “hidden sugars” and 20% as table sugar, which corresponds to ca. 24g of table sugar/d. Should the above mentioned dosage of natural sweeteners show positive metabolic effects, it seems a feasible dosage to replace daily direct sucrose intake. Xylitol is given in a dosage equisweet to erythritol.

2.4 Explanation for choice of comparator

We intend to use a control-group to compare effects. Unfortunately, a placebo-controlled study design is not possible in this case: a placebo would have to be approximately equisweet without any known metabolic effects. However, there are no such substances: artificial sweeteners such as e.g. aspartame are much sweeter and it would be impossible to keep the blinding. Apart from that, artificial sweeteners probably have metabolic side-effects and are not inert. All carbohydrates have some metabolic effects themselves. We accept the possible placebo-effect of the two polyols. However, participants will serve

as their own controls (before-after treatment) as well.

2.5 Risks / Benefits

Participants do not profit directly from this study. However, in the long term, findings could lead to a new nutritional concept in obese patients. In sensitive subjects consumption of polyols can lead to borborygmi and diarrhoea. However, if the amount of polyols is distributed over the day - as planned - we do not expect intake to cause discomfort. To gain peripheral venous access can be challenging in morbidly obese subjects. Occasionally, blood sampling can result in local skin irritation, hematoma and discomfort. However, well-trained study personnel (used to this special population) will place venous accesses and collect blood samples.

2.6 Justification of choice of study population

In this trial, obese but otherwise healthy volunteers will be included. In morbid obesity virtually all patients suffer from metabolic syndrome with non-alcoholic fatty liver disease, dyslipidaemia and impaired glucose tolerance. In animal models, xylitol consumption led to decrease in visceral fat[11] and metabolic syndrome. In a human study carried out on lean subjects in 1982, 3 weeks of xylitol intake showed a decrease in plasma cholesterol values[14].

3. STUDY OBJECTIVES

3.1 Overall Objective

The purpose of this study is to evaluate whether daily intake of erythritol or xylitol over a period of 7 weeks will alter gut microbiota composition and glucose tolerance.

3.2 Primary Objective

- Glycaemic control (fasting insulin & glucose & c-peptide after oral glucose challenge)

3.3 Secondary Objectives

- Human gut microbiota composition
- Metabolomics
- Blood lipids, hepatic enzymes
- Inflammatory response
- Intestinal permeability
- Gastrointestinal tolerance (bloating, diarrhoea etc.)
- Intestinal glucose absorption

3.4 Safety Objectives

The study also aims to assess tolerability in terms of incidence of gastrointestinal side effects of the two polyols.

4. STUDY OUTCOMES

4.1 Primary Outcome

- **Glycemic control:** Before and after treatment, an oral glucose tolerance test will be carried out. In this trial, we will include more time-points (0-30-60-90-120min. after glucose ingestion) for an accurate assessment of pharmacokinetics (AUC: area under the curve, Cmax, Tmax).

STANDARD ORAL GLUCOSE TOLERANCE TEST: DEFINITIONS
Diabetes: Fasting plasma glucose ≥7.0mmol/l or ≥11.1mmol/l (OGGT: 2–h plasma glucose))
Impaired Glucose Tolerance (IGT): Fasting plasma glucose <7.0mmol/l and ≥7.8 and <11.1mmol/l (OGGT: 2–h plasma glucose)
Impaired Fasting Glucose (IFG): Fasting plasma glucose 6.1 to 6.9mmol/l and <7.8mmol/l (OGGT: 2–h plasma glucose).
Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF consultation. World Health Organization 2006

4.2 Secondary Outcomes

- **Human gut microbiota composition:** Faecal samples will be taken before and after treatment and the taxonomic and functional profiles of the gut microbiota will be assessed by metagenomic shotgun sequencing.
- **Metabolomics:** Faecal and urine samples will be used for analysis of the metabolome using metabolomics.
- **Blood lipid profile, hsCRP and hepatic enzymes:** Fasting samples will be taken to measure triglyceride, total cholesterol, LDL and HDL levels, and the liver enzymes ASAT (aspartate transaminase) and ALAT (alanine transaminase). The analysis will be carried out in the hospital laboratory (St. Claraspital).
- **Inflammatory response:** As erythritol might act as a radical scavenger and reduce oxidative stress, we plan to assess inflammatory state by measuring IL-6, TNF-α, sTNFR1, sTNFR2, high sensitive CRP (hsCRP) and leucocytes.
- **Intestinal permeability:** Circulating markers of intestinal permeability (zonulin, LPS) will be measured.
- **Gastrointestinal tolerance (bloating, diarrhoea etc.):** We will assess gastrointestinal symptoms by using a validated questionnaire (Clinical rating scale for gastrointestinal symptoms: Svedlund et al.[21]). The questionnaire will be used at baseline and at the end of week 3 and 7 (self-reporting). A license agreement will be sent to AstraZeneca (ASTRAZENECA AB, 556011-7482 S-151 85 Södertälje, Sweden).

4.3 Intestinal Glucoseabsorption: We will assess intestinal glucose absorption by use of a validated technique: during the oral glucose tolerance test, 3g of 3-O-Methyl-Glucose (3-OMG) is ingested. 3-OMG is then measured in the plasma samples obtained at 0-30-60-90-120min. Other Outcomes of Interest

We accept that a possible confounder might be the substitution of sucrose in daily life as polyols might serve as a substitute. If sugar consumption is reduced, metabolic effects could be caused by reduced sugar exposure rather than polyol intake. We will therefore assess overall sugar consumption before and during the trial. Participants will keep a food diary. For this purpose we will use the clinical diary used by our nutritionists for preoperative assessment of bariatric patients.

4.4 Safety Outcomes

Gastrointestinal tolerance (bloating, diarrhoea etc.): Incidence and severity of gastrointestinal side effects related to the sweetener intake during the study is assessed by using a questionnaire (Clinical rating scale for gastrointestinal symptoms: Svedlund et al.[21])

5. STUDY DESIGN

5.1 General study design and justification of design

This pilot trial is a randomized, controlled interventional study; the project set-up will be single-centre, national. Participants will be randomized to one of the three groups: control-group (1), xylitol group (2) or erythritol group (3). They will then get pre-portioned sticks of either xylitol (24g/d; 3x8g) or erythritol (36g/d; 3x12g) or no food supplement (control-group). During the whole period patients will keep a food diary, where they will also report intake of sweeteners and once a week they will fill in a clinical gastrointestinal symptoms questionnaire. The food diary and gastrointestinal symptoms questionnaire will be sent to the clinical trial unit once a week. Once every week volunteers will receive a phone call by the study nurse in order to make sure the sweetener is well-tolerated, compliance is guaranteed and no adverse events occur. At baseline and after 7 weeks, patients will be examined: a) metabolic work-up: oral glucose tolerance test, liver enzymes, blood lipids, b) biomarkers of gastrointestinal permeability and inflammatory response, c) urine and faecal samples (metabolomics), and d) faecal sampling (gut microbiota composition). Upon conclusion of the study, remaining sweetener sticks and questionnaires are returned.

Demographic and personal data (year of birth, weight, height, age, gender, abdominal circumference, blood pressure, medical history) will be saved in a computer database.

Participant recruitment will be achieved through referring physicians from the Ernährungszentrum St. Claraspital, where morbidly obese patients are seen in an outpatient setting before bariatric surgery and are usually on a rather long waiting list before surgery. In case patients are interested in participating,

they will be contacted by the principal investigator (Dr: Bettina Wölnerhanssen) or the study nurse (Ms. Dorothea Brosi) and a screening appointment will be made.

5.2 Methods of minimising bias

Not placebo-controlled: We intend to use a control-group to compare effects. Unfortunately, a placebo-controlled study design is not possible in this case: a placebo would have to be approximately equisweet without any known metabolic effects. However, there are no such substances: artificial sweeteners such as e.g. aspartame are much sweeter and it would be impossible to keep the blinding. Apart from that, artificial sweeteners probably have metabolic side-effects and are not inert. All carbohydrates have some metabolic effects themselves. We accept the possible placebo-effect of the two polyols. However, participants will serve as their own controls (before-after treatment) as well.

Possible confounders: We accept that a possible confounder might be the substitution of sucrose in daily life as polyols might serve as a substitute. If sugar consumption is reduced, metabolic effects could be caused by reduced sugar exposure rather than polyol intake. We will therefore assess overall sugar consumption before and during the trial. Participants will keep a food diary.

Steps to reduce loss to follow up: We will call the volunteers once a week in order to achieve maximal compliance and minimize loss to follow-up.

5.2.1 Randomisation

The principal investigator Dr. Bettina Wölnerhanssen prepares a treatment list and enrolled participants are consecutively entered into this list. The treatment list is prepared beforehand by a (excel) computer-based randomization.

5.2.2 Blinding procedures

- The personnel performing analyses of faecal, urine and blood samples is blinded concerning the treatment group the participants belong to (control, xylitol, or erythritol).
- Control group: the study nurse, principal investigator and study participant are *not* blinded.
- Patients receiving either polyol are blinded as to which substance is used (xylitol or erythritol). The principal investigator and the study nurse are *not* blinded.

5.3 Unblinding Procedures (Code break)

Unblinding is permissible in case of an adverse event which results in premature study termination. The principal investigator is in possession of the key and will inform the participant in this case.

6. STUDY POPULATION

6.1 Eligibility criteria

Participants fulfilling all of the following inclusion criteria are eligible for the study:

- Obese volunteers (BMI > 30kg/m2) aged 18- max. 55 years
- Otherwise healthy.
- Informed Consent as documented by signature (Appendix Informed Consent Form)

The presence of any one of the following exclusion criteria will lead to exclusion of the participant:

- Known cardiovascular disease (coronary heart disease, cardiopathy, arrhythmia, pace-maker)
- Diabetes mellitus treated by oral antidiabetics or insulin
- Arterial hypertension with antihypertensive treatment
- Dyslipidaemia with statin therapy
- Known chronic hepatic disease (NASH, hepatitis). NAFLD is no exclusion criterion
- Known renal disease: kidney failure
- Pregnancy: although no contraindication for polyol intake, pregnancy might influence metabolic state and vascular function
- Women who are pregnant, Intention to become pregnant during the course of the study,
- Lack of safe contraception, defined as: Female participants of childbearing potential, not using and not willing to continue using a medically reliable method of contraception for the entire study duration, such as oral, injectable, or implantable contraceptives, or intrauterine contraceptive

devices, or who are not using any other method considered sufficiently reliable by the investigator in individual cases
- Chronical diseases of the gastrointestinal tract (also irritable bowel disease, food intolerance), history of gastrointestinal surgery with major changes to the gastrointestinal tract (removal of stomach, larger portions of the small bowel/colon, any bypass).
- Intake of prebiotics (such as inulin, oligofructose) on a regular basis
- Intake of probiotics (e.g. capsules with live bacteria or yeasts) on a regular basis
- Intake of pro/prebiotic food on a regular basis (e.g. Activia, Actimel, Aktifit, Yacult etc). Intake of common yoghurt is allowed, but limited to 1 serving/day
- Substance abuse, alcohol abuse
- Inability to follow procedures due to psychological disorders, dementia or insufficient
- Knowledge of project language (German).
- Participation in another study with investigational drug within the 30 days preceding and during the present study.

6.2 Recruitment and screening

Participant recruitment will be achieved through referring physicians from the Ernährungszentrum St. Claraspital, where morbidly obese patients are seen in an outpatient clinic before bariatric surgery and are usually on a rather long waiting list before surgery. In case patients are interested in participating, they will be contacted by the principal investigator (Dr: Bettina Wölnerhanssen) or the study nurse (Ms. Dorothea Brosi) per email, phone or face-to-face and a pre-screening is carried out (key questions to find out whether a volunteer is eligible). If the volunteer meets these key criteria, a screening appointment will be made and informed consent is sent by email or mail, so that the volunteer gets the chance to read through the information material before the screening visit.

6.3 Assignment to study groups

The principal investigator Dr. Bettina Wölnerhanssen prepares a treatment list and enrolled participants are consecutively entered into this list. The treatment list is prepared beforehand by a (excel) computer-based randomization and cannot be changed.

6.4 Criteria for withdrawal / discontinuation of participants

Participants might have to be excluded, in case of:
- Non-compliance
- Intolerable gastrointestinal effects (diarrhoea)
- Need for systemic antibiotic therapy
- Any other non-foreseeable adverse events

In case of discontinuation, already collected biological and health-related data will be used for analysis, except if the person concerned wishes to be excluded from analysis altogether. Encryption will be maintained at all times.

7. STUDY INTERVENTION

7.1 Identity of Investigational Products

7.1.1 Experimental Intervention (treatment / medical device)

Participants will be randomized to one of the three groups: control-group (1), xylitol group (2) or erythritol group (3). They will then get pre-portioned sticks of either xylitol (24g/d; 3x8g) or erythritol (36g/d; 3x12g) or no food supplement (control-group).

7.1.2 Control Intervention (standard/routine/comparator treatment / medical device)

The control-group receives no food supplement.

7.1.3 Packaging, Labelling and Supply (re-supply)

The two polyols xylitol and erythritol are given to the participants in form of sticks (such as sugar sticks) which are labelled with “Poly-Gut Study” and A or B. Sticks labelled with “A” contain 8g of xylitol and sticks labelled with “B” contain 12g of erythritol. The participant receives a whole batch of the respective portions for the whole study. This batch includes some extra portions, in case a portion is lost and in case the time gap at the end of the study has to be bridged until the final visit.

7.1.4 Storage Conditions

Polyols are temperature stable, but have to be stored in a dry space. After the study the remaining sticks are returned to the study personnel and are destroyed after counting.

7.2 Administration of experimental and control interventions

7.2.1 Experimental Intervention

1st week= adaption week; run-in: with increasing doses of sweetener:

During the first week, the participants assigned to one of the polyol-groups start with a low dose and then step up so that the gastrointestinal tract can adapt to the new substance. The whole portion is dissolved in some tap water (e.g. 1/2 dl or less) and ingested before the main meals (breakfast, lunch, dinner). In case the participant has forgotten one portion, he/she can take two portions at once without any problems.

- The first 2 days, the participant takes one portion per day (one portion in the morning)
- The next 3 days, the participant takes 2 portions per day (one portion in the morning, one at lunch)
- Then the participant takes 3 portions per day (one portion in the morning, one at lunch and one in the evening) till the end of the study (last visit).This last visit date will already be arranged at the beginning and will be max. 3 days after the 49 treatment days. The participants will get extra sweetener sticks in order to bridge the time gap (49+3 days).

7.2.2 Control Intervention

Participants assigned to the control-group get no sweetener, but will also go through all assessments.

7.3 Dose / Device modifications

In case the participant has forgotten one portion, he/she can take two portions at once without any problems. The participant cannot change the intake schedule and dosage. In case the polyol is not tolerated well (gastrointestinal symptoms), the study has to be discontinued.

7.4 Compliance with study intervention

Compliance is of uttermost importance in this project. To improve adherence to the intervention, participants are contacted by phone once a week by the study personnel. Apart from answering upcoming questions this allows the study personnel to remind the participant to take the sweeteners regularly, to fill in the questionnaires and to come to the appointments. At the end of the study, the return of unused portions will allow us to estimated compliance. Non-compliance: In case a participant does not take the sweeteners as planned, or fails to fill in the forms and come to the appointments the participant has to be excluded.

7.5 Data Collection and Follow-up for withdrawn participants

In case a participant wishes to discontinue study participation, the data collected to this time point is included in the analysis. A follow-up visit is not planned in this case.

7.6 Trial specific preventive measures

Concomitant medication with an antibiotic might impact findings in vascular assessment and needs to be reported to the study personnel.

7.7 Return or Destruction of sweeteners

The returned sweetener portions are counted and destroyed.

8. STUDY ASSESSMENTS

8.1 Study flow chart(s) / table of study procedures and assessments

See: STUDY SCHEDULE

8.2 Assessments of outcomes

8.2.1 Assessment of primary outcome

Oral glucose tolerance test (OGTT):
At baseline and after the treatment period (ca. 49d plus 3 days) an oral glucose tolerance test is carried out.

Patients are instructed:

- that they should not have been acutely ill (acute infection of any kind) within 14 days prior to OGTT,
- to abstain from strenuous exercise within the last 48h before OGTT,
- to make sure their main meals within the last 48h before OGTT include carbohydrates,
- that they must not eat or drink, nor consume alcohol or smoke within 10 hours before OGTT.

Blood sampling: After an overnight fast of at least 10 hours an antecubital catheter will be inserted into a forearm vein for blood collection. After a fasting blood sample on ice into 7.5mL tubes containing EDTA (6 µmol/L) the participant will ingest a 75g glucose solution (300 ml OGTT syrup, Roche) within 5-10min.

Further blood samples will be taken at regular time intervals (30, 60, 90, and 120 min).

Tubes will be centrifuged at 4º C at 3000 rpm for 10 min and 4 plasma sample aliquots are pipetted into 1.5mL tubes (aliquots à 600µL plasma each; tubes labelled: ① Insulin/Glucose, ② C-Peptide, ③ Reserve 1, ④ Reserve 2). Samples will be stored at -80º C until analysis of plasma glucose, insulin and C-peptide.

STANDARD ORAL GLUCOSE TOLERANCE TEST: DEFINITIONS
Diabetes Fasting plasma glucose ≥7.0mmol/l or ≥11.1mmol/l (OGGT: 2–h plasma glucose))
Impaired Glucose Tolerance (IGT) Fasting plasma glucose <7.0mmol/l and ≥7.8 and <11.1mmol/l (OGGT: 2–h plasma glucose)
Impaired Fasting Glucose (IFG) Fasting plasma glucose 6.1 to 6.9mmol/l and <7.8mmol/l (OGGT: 2–h plasma glucose)
- > OGTT: venous plasma glucose 2–h after ingestion of 75g oral glucose load
Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia: Report of a WHO/IDF consultation. World Health Organization 2006

8.2.2 Assessment of secondary outcomes

Faecal samples for human gut microbiota composition and metabolomics:

- Patients will get 2 faeces containers (107x25mm, Sarstedt, Ref. 80.622.111) for faecal sampling.
- One container will be used for gut microbiota analysis and one for metabolomics.
- Patients are instructed to pass the specimen (stool) onto a plastic wrap or disposable plate (at home) and to take ca. four pea-sized (or bigger) amounts of stool material for each plastic container using the spatula attached to its cap. Sampling needs to be from the internal side of the faeces (not just a swab of the surface).
- Patients will keep the sample in the fridge at 4ºC and bring them to St. Claraspital hospital within 24 hours, where the sample will be stored at -80ºC until analysis.

Analyses:

- The taxonomic and functional profiles of the gut microbiota will be assessed by metagenomic shotgun sequencing.
- Faecal samples will be used for analysis of the metabolome using metabolomics (using 1H-NMR 600mHz at UCL).[22]

Urine sample for metabolomics:

- The urine sample is freshly obtained (at our outpatient clinic, metabolic visit 1 and 2: after an overnight fast of 10 hours, but not necessarily first void in the morning).
- Patients receive a common urine container (as used in hospital) and are instructed to deliver midstream urine.

- From the freshly obtained urine, four aliquots of 1mL each are pipetted into Eppendorf's safe lock 2mL tubes and are frozen and stored at -80ºC until analysis.
- In case direct processing is impossible, urine is kept in the fridge at 4ºC for a maximum of 1 hour and then aliquoted and frozen.

Analyses:

- The urine samples will be used for analysis of the metabolome using metabolomics (using 1H-NMR 600mHz at UCL).[22]

Blood lipid profile, hepatic enzymes and hsCRP:

Fasting samples will be taken to measure triglyceride, total cholesterol, LDL (low-density lipoprotein) and HDL (high-density lipoprotein) levels, the liver enzymes ASAT (aspartate transaminase) and ALAT (alanine transaminase) and hsCRP (high-sensitive C-reactive protein) at baseline an after the treatment period. Analysis will be carried out in the hospital laboratory (St. Claraspital).

Reference values:

ASAT and ALAT:	< 35 U/L in females and < 50 U/L in males
Triglycerides:	< 2.0 mmol/L
Total cholesterol:	< 5.2 mmol/L
LDL:	< 2.6 mmol/L
HDL:	> 1.6 mmol/L
hsCRP:	< 5 mg/L

Experimental blood analysis:

For measurement of inflammatory response (IL-6, TNF-α, sTNFR1, sTNFR2), and intestinal permeability (zonulin, LPS), a fasting blood sample will be taken at baseline and after the treatment period.

Blood sampling: After an overnight fast of at least 10 hours, an antecubital catheter will be inserted into a forearm vein for blood collection. Venous blood samples are drawn into a sterile, pyrogen-free 7.5mL collection tube containing a clot activator and gel for serum separation and then centrifuged at 3000 rpm for 10 min at 4°C. In addition, for LPS, pyrogen free heparin citrate tubes are used in venipuncture.

Aliquots: Six serum aliquots (containing 600µL serum each; labelled ① IL-6, ② TNF-α, ③ sTNFR1, ④ sTNFR2, ⑤ Zonulin ⑥ Reserve experimental) are pipetted with pyrogen-free tips into 6x1.5-ml pyrogen-free tubes and frozen at −80°C until further analysis. In addition, two serum aliquots (containing 250µL serum each; labelled ① LPS-1, ② LPS-2) are pipetted with pyrogen-free tips into 2x1.0-ml pyrogen-free tubes and frozen at −80°C until further analysis.

Inflammatory response

- High sensitive CRP (hsCRP) is measured in the hospital laboratory (St. Claraspital), see above.
- For IL-6 and markers of the tumour necrosis factor α pathway [TNFα, soluble TNF receptor 1 (sTNFR1), and 2 (sTNFR2)] aliquots are stored:

Analyses:

- **Serum IL-6** will be measured by a commercially available ELISA kit (EZHIL6; Merck-Millipore)
- **Serum TNF-α** levels will be measured using a commercially available enzyme-linked immunosorbent assay (Invitrogen KHC 3011, U.S.A.). Results are expressed as pg/mL.
- **Serum sTNFR1** levels will be measured using a commercially enzyme-amplified sensitivity immunoassay kit (Invitrogen, KAC 1761, U.S.A.). Results are expressed as ng/mL.
- **Serum sTNFR2** levels will be measured using a commercially enzyme-amplified sensitivity immunoassay kit (Invitrogen, KAC 1771, U.S.A.). Results are expressed as ng/mL.

Intestinal permeability:

- **Serum zonulin** concentrations will be measured by a zonulin ELISA Kit (K5600, Immundiagnostik AG, Bensheim, Germany).

- **LPS** (lipopolysaccharide) concentrations will be measured by LAL (Limulus amebocyte lysate) technique (MCS cartridges Charles River, U.S.A.).

Gastrointestinal tolerance (bloating, diarrhea, reflux):

Gastrointestinal symptoms will be assessed by use of a validated questionnaire (Clinical rating scale for gastrointestinal symptoms: Svedlund et al.,[21]). The questionnaire will be used at baseline and at the end of week 3 and 7 (self-reporting). A license agreement will be sent to AstraZeneca (ASTRAZENECA AB, 556011-7482 S-151 85 Södertälje, Sweden). The GSRS is a disease-specific instrument, developed based on reviews of gastrointestinal symptoms and clinical experience, to evaluate common symptoms of gastrointestinal disorders.[21,23] The GSRS contains 15 items, each rated on a seven-point Likert scale from no discomfort to very severe discomfort. Based on a factor analysis, the 15 GSRS items break down into the following five scales: abdominal pain (abdominal pain, hunger pains and nausea); reflux syndrome (heartburn and acid regurgitation), diarrhoea syndrome (diarrhoea, loose stools and urgent need for defecation), indigestion syndrome (borborygmus, abdominal distension, eructation and increased flatus) and constipation syndrome (constipation, hard stools and feeling of incomplete evacuation).[23] The scores are calculated by taking the mean of the items completed within an individual scale, with higher scores indicating greater severity of symptoms. The GSRS in European patient populations has a good internal consistency reliability and acceptable construct validity and responsiveness.[23-25] The GSRS can be administered in either self-report or interview format.

Intestinal Glucoseabsorption: We will assess intestinal glucose absorption by use of a validated technique [26,27]. During the oral glucose tolerance test, 3g of 3-O-Methyl-Glucose (3-OMG) is ingested. 3-OMG is then measured in the plasma samples obtained at 0-30-60-90-120min by means of high performance exchange chromatography.

8.2.3 Assessment of other outcomes of interest

Food diary: In order to minimize the confounding factor of possible reduced sugar intake, prior to inclusion nutrition habits will assessed by a questionnaire. Participants will also fill in a food diary during week 3 and 7 (see appendix).

8.2.4 Assessment of safety outcomes

Participants are contacted on a weekly base. However, they will all be provided with a personal phone number of the principal investigator Dr. Bettina Wölnerhanssen and the study nurse Ms. Dorothea Brosi and in case of any undesired effects, participants are to inform the principal investigator directly.

8.2.4.1 Adverse events

Possible adverse events associated with polyol-intake are gastrointestinal symptoms (diarrhoea, bloating), therefore we will assess gastrointestinal symptoms before and during the study by means of a questionnaire (GSRS). In theory an allergic reaction to one of the two substances are also possible. Participants will be asked to report any specific and unspecific symptoms.

8.2.5 Assessments in participants who prematurely stop the study

Participants who discontinue the study are not seen for a follow up visit, as the data obtained after discontinuation will not be useful for our analysis. However, the baseline data will be included in our analyses.

8.3 Procedures at each visit

8.3.1 First Visit = Screening

The **first visit (screening visit)** at our outpatient clinic will take about 60min. We will explain the study and will make sure the candidate is eligible for this trial. The candidate must arrive on an empty stomach (not allowed to eat or drink 10 hours prior to this appointment except for tap water or unsweetened herbal tea without milk).

- We will ask questions about his/her general health and will explain the study in detail. We will also briefly examine the candidate (measurement of blood pressure, weight).
- In women of childbearing age (18-49 years old), who do not take any contraceptives we will carry out a urine pregnancy test.
- We will take a blood sample and examine HbA1C, creatinine, ALAT, ASAT, hsCRP and blood lipids.
- The participant will receive a faecal sample kit with detailed instructions on how to take a stool sample.

- The participant will receive a study folder with detailed instructions for the whole study. Included in this study folder is a food diary, which the participant will be asked to fill out during a 7 day period (before starting with the intake of the substance).

8.3.2 Second Visit = 1. Metabolic Visit

The **second visit (1. metabolic visit)** at our outpatient clinic will take about 2.5 hours. We will carry out an oral glucose tolerance test and take blood, and urine samples. If possible, the participant will bring the first faecal sample to this visit. Again, the participant has to arrive on an empty stomach (not allowed to eat or drink 10 hours prior to this appointment except for tap water or unsweetened herbal tea without milk). Even chewing gum must be avoided, as they might contain sugar. In addition 2 days prior to the test the participant has to refrain from heavy physical activity and needs to have carbohydrates as part of her/his meals.

- The candidate will be assigned randomly to one of the three groups (intake of xylitol, or erythritol or control group).
- The participant needs to bring his/her study folder along, including the food diary which he/she should have kept over the course of 1 week.
- If possible, the participant will bring along the first faecal sample
- We will take a first blood sample to measure blood sugar, insulin, C-peptide (for the oral glucose tolerance test). In addition we will measure biomarkers of inflammatory response (IL-6, TNF-α, sTNFR1, sTNFR2) and intestinal permeability (zonulin and LPS).
- The participant will receive 75 g of glucose (grape sugar) plus 3g 3-O-Methyl-D-glucose dissolved in 300mL tap water which he/she will have to drink over a period of 5-10min. („oral glucose tolerance test")
- After 30, 60, 90 and 120min we will again take blood samples for measurement of blood sugar, insulin, C-peptide and 3-OMG. In total we will take a maximum of 30mL blood (corresponding to 3 tablespoons).
- During this appointment we will fill in the questionnaire for gastrointestinal symptoms together with the participant.
- At the end of the visit, the participant will receive the portioned sweeteners (if assigned to one of the polyol groups) and the kit for the second faecal sample.

8.3.3 Intake of polyols

Intake of sweetener: After the first two appointments; after taking blood and the first faecal and urine sample, and after filling in the questionnaire for gastrointestinal symptoms and keeping the food diary over 7 days the participant will be ready to start with the sweetener intake. Participants assigned to the control group receive no sweetener.

8.3.4 Follow-up appointments

Appointment: The follow-up visit (3rd visit) will take place in the eighth week. As it can be difficult to make an appointment directly on the first day of week 8, we will provide the participant with some extra sweetener portions to bridge the gap until the visit. There shouldn't be more than 3 extra days. The participant needs to continue with the sweetener intake until the last visit.

8.3.5 Third Visit = 2. Metabolic visit

The **third visit (2. metabolic visit)** at our outpatient clinic will take about 2.5 hours.
We will carry out an oral glucose tolerance test and take blood and urine samples. Again, the participant has to arrive on an empty stomach (not allowed to eat or drink 10 hours prior to this appointment except for tap water or unsweetened herbal tea without milk). Even chewing gum must be avoided, as they might contain sugar. In addition 2 days prior to the test the participant has to refrain from heavy physical activity and needs to have carbohydrates as part of her/his meals.

- The participant must bring along his/her study folder, the remaining sweetener portions and the second stool sample if possible.
- We will briefly examine the participant (measurement of blood pressure, weight).
- We will take a first blood sample to measure blood sugar, insulin, C-peptide (for the oral glucose tolerance test). In addition we will measure biomarkers of inflammatory response (hsCRP, IL-6, TNF-α, sTNFR1, sTNFR2) and intestinal permeability (zonulin and LPS) and measure ALAT, ASAT, blood lipids and leucocyte count.

- The participant will receive 75 g of glucose (grape sugar) plus 3g 3-O-Methyl-D-glucose dissolved in 300mL tap water which he/she will have to drink over 5-10min. („oral glucose tolerance test").
- After 30, 60, 90 and 120min we will again take blood samples for measurement of blood sugar, insulin, C-peptide and 3-OMG levels. In total we will take a maximum of 30mL blood (corresponding to 3 tablespoons).

SCHEDULE OF ASSESSMENTS (FLOW OF RESEARCH PROJECT)

Project Periods	**Screening Visit (V1)**	**Baseline Metabolic Visit (V2)**	**Intake of sweetener:** • erythritol or xylitol • week 1: adaption • week 2-7: 3 x/day							**Follow-up Metabolic Visit (V3)**
Location	St. Claraspital	St. Claraspital								St. Claraspital
Time (hour, day, week)	-7d	0d								49d +max. 3 days
			Weeks							
			1	2	3	4	5	6	7	
Participant Information and Informed Consent	x		**Adaption week with increasing doses:** 2 days 1 portion (1-0-0) 3 days 2 portions (1-1-0) 2 days 3 portions (1-1-1) • • •							
Demographics: weight, height, abd. circumference	x									x
Medical History	x									
In- /Exclusion Criteria	x									
Randomization		x								
Fasting blood sample	x	x								x
• Haemoglobin	x									
• Leucocytes	x									x
• HbA1C	x									
• Liver enzymes: ALAT, ASAT	x									x
• Creatinine	x									
• Lipid panel (Triglyc., total Chol., HDL, LDL)	x									x
• hsCRP	x									x
• Serum aliquots for: - IL-6, zonulin - TNF-α - sTNFR - sTNFR2 - LPS		x								x
Urine Pregnancy Test	x									
Oral glucose tolerance test 75g glucose (0-30- 60-90-120min)		x								x
• Blood glucose		x								x
• Insulin		x								x
• C-peptide		x								x
• 3-O-Methyl-D-Glucose		x								x
Urine sample		x								x
Faecal sample		x								x
GSRS: questionnaire gastrointestinal symptoms	x				x				x	
Food diary		x			x				x	
Follow-up calls				x	x	x	x	x	x	
SAE				x	x	x	x	x	x	x
End of study meeting										x

9. SAFETY

During the entire duration of the study, all adverse events (AE) and all serious adverse events (SAEs) are collected, fully investigated and documented in source documents and case report forms (CRF). Study duration encompassed the time from when the participant signs the informed consent until the last protocol-specific procedure has been completed, including a safety follow-up period.

9.1.1 Definition and assessment of (serious) adverse events and other safety related events

An **Adverse Event (AE)** is any untoward medical occurrence in a patient or a clinical investigation participant administered a pharmaceutical product and which does not necessarily have a causal relationship with the study procedure. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product. [ICH E6 1.2]

A Serious Adverse Event (SAE) is classified as any untoward medical occurrence that:

- results in death,
- is life-threatening,
- requires in-patient hospitalization or prolongation of existing hospitalisation,
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect.

In addition, important medical events that may not be immediately life-threatening or result in death, or require hospitalisation, but may jeopardise the patient or may require intervention to prevent one of the other outcomes listed above should also usually be considered serious. [ICH E2A]

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

SAEs are followed until resolution or stabilisation. Participants with ongoing SAEs at study termination (including safety visit) will be further followed up until recovery or until stabilisation of the disease after termination.

Assessment of Causality

Both Investigator and Sponsor-investigator make a causality assessment of the event to the test substance, based on the criteria listed in the ICH E2A guidelines:

Relationship	Description
Definitely	Temporal relationship Improvement after dechallenge* Recurrence after rechallenge (or other proof of drug cause)
Probably	Temporal relationship Improvement after dechallenge No other cause evident
Possibly	Temporal relationship Other cause possible
Unlikely	Any assessable reaction that does not fulfil the above conditions
Not related	Causal relationship can be ruled out
*Improvement after dechallenge only taken into consideration, if applicable to reaction	

9.1.2 Reporting of serious adverse events (SAE) and other safety related events

All SEs are documented in the participants´ file and on the SE report form. The project leader shall report any occurring SE to the responsible EC within 7 days. She shall also submit a report which evaluates the relationship between the event reported and the methods of collecting health related personal data or sampling of biological material within that project, furthermore proposals how to proceed with the project. The project leader shall notify the EC within 7 days of any immediate other safety and protective measures, which have to be taken during the conduct of the research project. In

addition, the project leader shall explain the circumstances, which necessitated the safety and protective measures.

Reporting of SAEs
All SAEs must be reported immediately and within a maximum of 24 hours to the Sponsor-Investigator of the study. The Sponsor-Investigator will re-evaluate the SAE and return the form to the site.
SAEs resulting in death are reported to the local Ethics Committee (via local Investigator) within 7 days.

Reporting of SUSARs
A SUSAR needs to be reported to the local Ethics Committee (local event via local Investigator) and to Swissmedic for category B and C studies (via Sponsor-Investigator) within 7 days, if the event is fatal, or within 15 days (all other events).

Handling of Pregnancies
Pregnant participants must immediately be withdrawn from the clinical study. Polyol intake is not contraindicated in pregnancy, as this food additive is generally regarded as safe. However, pregnancy might influence our findings

Periodic reporting of safety
An annual safety report is submitted once a year to the local Ethics Committee.

9.1.3 Follow up of Adverse Events
In case of gastrointestinal symptoms, discontinuation of polyol intake will immediately alleviate symptoms. The participant will be told which test substance he/she had taken to avoid future discomfort (deblinding) and will be advised to consult her/his family practitioner.

10. STATISTICAL METHODS

10.1 Hypothesis
In this exploratory pilot study a hypothesis testing is non-applicable. We expect that glycaemic control is ameliorated in obese, non-diabetic (but insulin resistant) patients compared to baseline, which might be explained through favourable alterations in gut microbiota composition. Should we be able to find an effect, this pilot trial might serve as a basis for further studies including more patients

10.2 Determination of Sample Size
In each group we will examine 20 volunteers. This is a pilot trial and findings will serve to plan larger studies in the future. At this moment no power analysis is possible and group size is chosen on the basis of practical considerations rather than statistical estimation. However, according to our experience, a sample size of min. 20 subjects in each arm will most likely allow us to detect large differences in parameters (> 50%) between the treatments groups.

10.3 Statistical criteria of termination of trial
N/A.

10.4 Planned Analyses

10.4.1 Datasets to be analysed, analysis populations
In this pilot trial which is purely observational, an analysis per protocol is planned. Drop-outs will be replaced, but baseline data of drop-outs might be included to characterize the baseline population. Baseline values of all three groups will be compared, to rule out any baseline differences. The control group will also be examined twice, and the two time-points should yield similar results. Data from each participant before and after treatment will be analyzed in the sweetener groups. In that way, each participant will serve as his or her own control. Post-treatment values of both treatment groups will then be compared to the control group (without treatment).
In case both sweeteners should have an effect on our endpoints, we might combine the two arms for an additional analysis comparing “no sweetener” (n= 20) vs. “sweetener” (n= 40).

10.4.2 Primary Analysis

Descriptive statistics will be used for demographic variables, such as age, weight, height and BMI. Hormone and glucose profiles will be analyzed by calculating the area under the concentration-time curve (AUC) from baseline values. The parameters will be tested for normality by the Shapiro-Wilk test method. One-way ANOVA will be applied to describe differences between the groups. Student's paired t-test will be used to test for significant differences between subjects before and after sweetener intake. All statistical analysis will be done using the statistical software package, SPSS for Windows, Version 22.0 (SPSS Inc., Chicago, USA). Values will be reported as mean ± SEM. Differences will be considered to be significant when $P < 0.05$. Statistics will be done by the principal investigator with support of Prof. J. Drewe, biostatistician.

10.4.3 Secondary Analyses

No secondary analyses or subgroup analysis is planned.

10.4.4 Interim analyses

We do not plan any interim analysis.

10.4.5 Safety analysis

Any advert events are listed and treatment groups are compared.

10.4.6 Deviation(s) from the original statistical plan

Any deviations from the original statistical plan will be reported and justified in the final report.

10.5 Handling of missing data and drop-outs

Missing data: as a basic principle, we will only analyse available data. In case of single missing blood values, a "last observation carried forward" (LOCF) approach will be used. Drop-outs will be replaced. Data from drop-outs will be included in the analysis if feasible (e.g. baseline values can be of value to characterize the study population).

11. QUALITY ASSURANCE AND CONTROL

11.1 Data handling and record keeping / archiving

Personal data is kept in a computer data base, and all case report forms and informed consents are kept in a folder and archived for a minimum of 10 years.

11.1.1 Case Report Forms

Study data is recorded with paper Case Report Forms. For each enrolled study participant CRFs are maintained. Participants are not identified in the CRF by name or initials and birth date; instead the participant number is used.
The study nurse and the principal investigator are authorized for all CRF As paper CRFs are used, the data is entered into an electronic database for analysis at the end of the trial (by the study nurse and/or a master student).

11.1.2 Specification of source documents

Demographic data, visit dates, participation in study and Informed Consent Forms, randomisation number, SAEs, AEs and concomitant medication, results of relevant examinations and all CRFs are considered the source documents in the study. Source documents are archived in folders at the study site (St. Claraspital, Forschungsabteilung).

11.1.3 Record keeping / archiving

All study data will be archived for a minimum of 10 years after study termination or premature termination of the clinical trial. All data is archived in folders at the study site (St. Claraspital, Forschungsabteilung).

11.2 Data management

11.2.1 Data Management System

Data is entered in a computer data base (excel).

11.2.2 Data security, access and back-up

The database will be open to all study personnel involved in this trial: Prof. Christoph Beglinger, Ms Dorothea Brosi, Dr. phil. Anne Christin Meyer Gerspach, and the master student in charge: non nominatus. As it is stored on the hospital server, regular back-ups are made.

11.2.3 Analysis and archiving

Upon conclusion, the database is secured and cannot be changed anymore. The excel data base is also printed out and stored in a study folder.

11.2.4 Electronic and central data validation

Selected variables are checked through double entry.

11.3 Monitoring

N/A.

11.4 Audits and Inspections

Audits and inspections are not planned.

11.5 Confidentiality, Data Protection

Direct access to source documents will be permitted for purposes of monitoring, audits and inspections. All study personnel involved in this trial (Prof. Christoph Beglinger, Ms Dorothea Brosi, Dr. phil. Anne Christin Meyer Gerspach, Prof. Arno Schmidt-Trucksäss and the master student in charge: non nominatus) will have access to protocol, dataset during and after the study (publication, dissemination). Demographic data and personal data will be kept in the electronic data base. Subjects will receive a study number upon inclusion, and in the data base only the study number will appear. Only the principal investigator (Dr. Bettina Wölnerhanssen) will have the key.

Data generation, transmission, storage and analysis of health related personal data and the storage of biological samples within this project will follow strictly the current Swiss legal requirements for data protection and will be performed according to the Ordinance HRO Art. 5.

Health related personal data captured during this project and biological samples from participants are strictly confidential and disclosure to third parties is prohibited; coding will safeguard participants' confidentiality.

Project data shall be handled with uttermost discretion and only be accessible to authorised personnel. Ubiome® (www. ubiome.com) will provide us with raw data, which we will correlate with other findings (such as glucose tolerance). Participants will need to register at Ubiome®. This company guarantees full anonymity. Participants can choose, whether their data can be used for other studies or not.

11.6 Storage of biological material and related health data

Biological samples (blood samples) are stored in the freezer at St. Claraspital at -70°C until analysis. After a maximum of 10 years, the samples are destroyed. The freezer is connected to an alarm system.

12. PUBLICATION AND DISSEMINATION POLICY

We plan to publish the results in a peer-reviewed scientific journal. Upon request, we will provide the full study protocol and data (as required by some journals). The trial results might be presented at scientific congresses. No use of professional writers is intended. The principal investigator will have ultimate authority over any of the activities.

12.1 Funding

This project is part of a research grant application to the Swiss National Science Foundation (March 2016; answer pending). There is no conflict of interest and the financing party has no influence on the protocol, analysis or publication.

13. INSURANCE

The St. Claraspital will guarantee insurance (Basler Versicherungen, Aeschengraben 21 Postfach 2275, CH-4002 Basel).

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15. APPENDICES

CRF: Pre-Screening; CRF: Screening; CRF: Metabolic assessment 1&2; Study folder with: Intake protocol, food diary and Gastrointestinal Symptoms Rating Scale Questionnaire.