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Protocol for acute and repeated dose impact of sweeteners and sweetness enhancers on appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-over, randomised, controlled trial in people with overweight/obesity. The SWEET project.

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Abstract

Introduction: Intake of free sugars in European countries is high and attempts to reduce sugar intake have been mostly ineffective. Sweeteners and sweetness enhancers (S&SEs) can maintain sweet taste in the absence of energy, but little is known about the impact of acute and repeated consumption of these products on appetite. This study aims to evaluate the effect of acute and repeated consumption of 2 individual S&SEs and 2 S&SE blends in semi-solid and solid foods on appetite and related behavioural, metabolic and health outcomes.

Methods and Analysis: A work package of the SWEET project, this study consists of 5 double-blind randomised cross-over trials which will be carried out at 5 sites across 4 European countries, aiming to have n=213. Five food matrices will be tested across 3 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends and no added sugar). Participants (body mass index (BMI) 25-35 kg/m^2; aged 18-60 years) will consume each formulation for 14 days. The primary endpoint is composite appetite score (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour postprandial incremental area under the curve during clinical investigation days on day 1 and 14.

Ethics and dissemination: The trial will be approved by national ethical committees before starting recruitment and will be conducted in accordance with the Declaration of Helsinki. Results will be published in international peer-reviewed open access scientific journals. Research data from the trial will be deposited in an open access online research data archive.

Trial registration number: Clinicaltrials.gov: NCT04633681

Strengths and Limitations of this study:

- The trial is the first of its kind to investigate the effects of acute and repeated exposure to 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary (chocolate) and breakfast cereal.
- This trial includes a large range of outcomes across behaviour, physiology, and health from persons living in Northern, Central and Southern Europe.
- The COVID-19 pandemic resulted in changes to the design of the studies in the trial. Originally, all products were to be tested across 2 sites, but the reduced time frame means this is not possible for some products.

- Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will be reported descriptively in these 2 studies where appropriate.

BACKGROUND AND RATIONALE

The global increase in the prevalence of obesity and its associated diseases is driven by a range of internal factors, involving genetic, behavioural, and metabolic determinants along with permissive external factors from the physical, social, and political environment (1). Two of the main behavioural drivers involve a diet too rich in energy and lack of sufficient physical activity. Free sugar intake (derived from sugar added to foods and beverages by the manufacturer or consumer) is one nutritional component that has gained focus because of its low nutritional value (lack of vitamins, minerals or fibre) and its potential to add to the overall energy density of diets, facilitating weight gain (2). In 2015, the World Health Organisation (WHO) published a report with a specific focus on sugar intake and strongly recommended that free sugar intake should constitute <10 % of total daily energy intake (E%) and preferably <5 E% as a conditional recommendation (2). This is a recommendation that is largely unaddressed in Europe (3). The replacement of free sugars with sweeteners and sweetness enhancers (S&SEs) in food products is one method to reduce sugar intake while maintaining acceptance and palatability of the diet. S&SEs have increasingly been employed over recent years to reduce the energy and sugar content of foods; however, their impact on appetite- and health-related outcomes is somewhat unclear (4).

The effect of S&SEs on appetite is difficult to summarise due to the number of S&SEs available and the different types of studies and comparisons used. It is likely that different S&SE will have different modes of action and findings in one S&SE does not necessarily translate to all S&SEs. A recent review comparing different S&SEs suggests that some have the potential to enhance appetite, but these effects do not follow through to subsequent energy intake (4). A recent meta-analysis detailed the impact of no- or low-energy sweetened preloads compared to conventionally sweetened preloads on *ad libitum* energy intake. They concluded that similar effects on energy intake were seen due to only partial compensation being evident (although total energy intake was lower in the no- or low-energy sweetener) (5). Furthermore, recent studies have highlighted that S&SEs may reduce sweet food cravings and therefore reduce sugar intake (6)appetite and energy intake (7). Overall, there is currently insufficient evidence to make a clear conclusion about the effect of S&SEs on

appetite and energy intake. This is particularly true when discounting beverage studies, as there is a lack of information regarding S&SEs in semi-solid and solid foods. Furthermore, it should be acknowledged that differences between acute and longer-term effects of S&SEs may not be the same (8).

One of the reasons for the current partial understanding of the appetitive and metabolic effects of S&SEs in humans is that different S&SEs are commonly assumed to have similar behavioural effects (4, 9, 10). Only recently, one 12-week investigation of 4 distinct S&SEs reported directionally dissimilar effects of saccharin compared to sucralose on body weight (11). Indeed, the 11 S&SEs that are currently approved for use in the EU are chemically heterogeneous and absorbed, metabolised, and excreted differently (12). Furthermore, most investigations of the relationship between S&SE intake and health outcomes have used beverages as the vehicle (13) ; these have recently been reviewed (14). Since the amount of S&SEs in the food supply is increasing in response to consumer demand (15) and policy (e.g., 'sugar taxes'(16, 17); EU initiatives (18, 19)), there is a pressing need to examine the appetite-related behavioural and metabolic consequences of consuming S&SEs particularly in semi-solid and solid food matrices.

AIMS AND OBJECTIVES

The main objective of this study is to evaluate the acute (1-day) and repeated (14-day) effects of 2 individual S&SEs and 2 S&SE blends in reformulated reduced or no added sugar food products (using 2 modulations of S&SEs per matrix) on appetite and related behavioural, metabolic and health outcomes in adult men and women with overweight or obesity.

The hypotheses are:

H_1 Consumption of no added/reduced sugar products reformulated with S&SEs will result in an altered incremental area under the curve (iAUC) appetite score, compared with the sucrose-sweetened control product after repeated compared with acute consumption.

H_2 There will be differences between the no added/reduced sugar and sucrose-sweetened formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-related (liver function and gastrointestinal (GI) side effects) outcomes.

TRIAL DESIGN

This study is part of the SWEET project (Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability -Master protocol WP2 Phase 2 Version 2.6 29-MARCH-2022). It is a multicentre double-blind, randomised cross-over trial conducted across 5 intervention sites in 4 countries, with 3 product formulations (sucrose-sweetened control vs 2 individual S&SEs or S&SE blends) over 5 intervention product types (cake, biscuits, yoghurt, chocolate, and breakfast cereal) aiming for a total of 213 completers: Biscuit matrix n=52; yoghurt matrix n=54; chocolate matrix n=54; cereal matrix n=30; cake matrix n=24. The protocol is reported as per the SPIRIT reporting guidelines (20).

Sample Size Determination

The following calculations apply to the studies involving biscuit, yoghurt and chocolate matrices:

Primary outcome: Power calculations showed that to detect a minimum difference of 8 mm in appetite ratings on a 100 mm visual analogue scale (VAS) with 80% power, alpha 0.05, and based on a published within-subject SD of 14.4 mm in VAS measures (21), an overall sample of 53 completers (both sexes, same BMI group, across all centres) would be needed (22; p.30) per matrix.

Secondary outcomes:

- Blood glucose - choosing 6.5 mmol/l as the blood glucose peak in the control condition, a hypothesised 30% reduction for the S&SE conditions (4.55 mmol/L) and based on an expected SD of 2.0 (23) an effect size (d_z) of 0.975 can be calculated using alpha of 0.01 (to account for multiple comparisons) and a two-tailed distribution. With these data the minimum sample size is of 16 completers.
- Gut hormones - the minimum sample size for gut hormones was estimated based on values for the pancreatic polypeptide (PP) (15' peak), according to published data (24). These suggested a minimum of 30 completers per matrix were needed with 80% power, alpha 0.05 and a strong (0.9) effect size. It was estimated that this sample size would also suffice to detect differences in plasma ghrelin in participants with overweight (25).

Due to the impact to the trial brought about by the COVID-19 pandemic, the cake and breakfast cereal studies had to be scaled down and the study using cakes will no longer include blood

samples. The primary outcome will be reported as descriptive statistics only in these 2 studies where appropriate and reflected in the study registration and protocol.

STUDY SETTING

This trial is conducted across 5 intervention sites in 4 countries across 3 regions of Europe. Western Europe: Leeds (University of Leeds, UK); Liverpool (University of Liverpool, UK); Lyon (Centre de Recherche en Nutrition Humaine Rhône Alpes, France). Northern Europe: Copenhagen (University of Copenhagen, Denmark). Southern Europe: Pamplona (University of Navarra, Spain). University's of Leeds and Navarra are the leaders of this work package, whilst University of Liverpool is the co-ordinating centre of the SWEET project in its entirety.

PATIENT AND PUBLIC INVOLVEMENT

During the study, research staff discuss with participants about their experiences of the clinical investigation days, examinations, participant information and written materials, etc. with the aim to understand and improve participants' experiences in current and future studies of this nature. This is also captured in an end of study survey.

ELIGIBILITY CRITERIA

Male and female adults aged 18-60 years, with a BMI 25-35 kg/m^2 are eligible. Participants are required to regularly consume sugar-containing foods and willing to consume sugar and sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-26) (26) score <20 and a short sweet food frequency questionnaire score ≥3 of 11, in addition to rating the control product as ≥40% on a 100-point liking VAS during the taste test and be willing to consume the product during the duration of the trial. All exclusion criteria are listed in **Supplemental Material 1**.

INTERVENTION

Each trial will begin with an initial exposure to one of the 3 assigned product formulations under controlled laboratory conditions (clinical investigation day, CIDs 1, 3, 5 – exposure day 1), followed by repeated daily consumption of the same product at home for 12 (± 2 days) and a final exposure in the laboratory on day 14 (± 2 days) under identical conditions as the first exposure (CIDs 2, 4, 6 – exposure day 14), resulting in all participants completing

the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be followed by a wash out period of 14-21 days between formulations. During the at-home periods, participants will consume a portion of the product at a time and place they choose using a substitution strategy for similar energy/sweetness foods in their habitual diet. This strategy is supported by advice and agreement from the research officer/dietitian. Compliance will be monitored by an intervention booklet completed daily and by return of empty food packaging. All food products are provided in the same blinded container/wrapping. The study duration for each participant will be a minimum of 70 days (plus 7-14 days allowance for extended washout to aid scheduling of CIDs).

Recruitment and Screening

1) Participants will be recruited via a variety of routes e.g., study databases, webpages, social media, posters, and flyers. Potential participants will be pre-screened using an online or telephonic pre-screening questionnaire in accordance with the inclusion and exclusion criteria. Candidates passing pre-screening will be invited to attend an information session, either online or in-person, where they will be given detailed information about the study and invited to participate in a Q&A session. Candidates who wish to participate in the study will provide written informed consent and sign a general data protection regulation (GDPR) form before being fully screened. The screening session will be performed in-person or online, and will consist of anthropometric measurements (height, weight, waist and hip circumference; all confirmed in-person at CID1 for participants being screened online); eligibility questionnaires (EAT-26 (26) and short sweet food frequency questionnaire); baseline questionnaires (A socio-demographic questionnaire, a questionnaire to assess habitual sweet food consumption, including regular and S&SE sweet foods (SWITCH sweet food frequency questionnaire (SWFFQ)) (27), a questionnaire to assess habitual physical activity (International Physical Activity Questionnaire (IPAQ)) (28) and a consumer perspective questionnaire); an eligibility taste test of the control intervention product. Candidates who pass the screening session will be enrolled into the study.

Randomisation and blinding

A Latin square design (6 treatment orders) will be used to randomly allocate product sequence into blocks of 6, as shown in Figure 1. The person responsible for generating the

sequences for all sites will not have any study related tasks e.g., inclusion or examination of participants. Each sequence will be stratified by sex (female/male) and age group (18-45 years/46-60 years). When feasible, a female/male ratio of minimum 60/40 was also considered to reflect the target population characteristics.

Blinding of the intervention products (reformulated and control products) will be done by the manufacturers. As such, blinding of the research and central laboratory staff will take place allowing for a double-blind intervention. Moreover, the statistical analyses of the main outcome variable will be done without breaking the intervention product-assignment code before the analyses are finalised.

[Figure 1]

Clinical investigation days

Prior to each CID, participants will be asked to consume a similar evening meal at the same time, before fasting for a minimum of 12 hours and a maximum of 15 hours. High intensity physical activity, alcohol, and coffee will not be allowed for 12 hours before arriving to the laboratory. Two glasses, approximately 500 mL, of non-carbonated water will be allowed during the fasting period. Participants will provide a spot urine sample collected max 24 hours before each CID and will be analysed for the presence of specific S&SEs.

The CID procedures are outlined in Figure 2. CID start times will be scheduled in the morning between 8.00 am and 10.30 am and participants will start all 6 CIDs at the same time. Participants will complete a protocol compliance questionnaire to verify the above requirements regarding diet, physical activity, etc. If compliance has been breached, staff will reschedule the CID (within the maximum 14 days allowed, otherwise a protocol deviation will be recorded). If compliance has been achieved, participants will then fill in the Control of Eating Questionnaire (CoEQ)(29) to assess cravings over the last 7 days, followed by a body weight measurement. Participants will consume 200 mL of water before having an intravenous cannula inserted into an antecubital vein by qualified personnel. A baseline fasting blood sample will be taken 15 minutes after insertion of the cannula. Once the fasting sample has been taken, participants will complete fasting subjective appetite ratings for hunger, fullness, thirst, desire to eat, prospective intake, nausea, bloating, appetite for

something savoury and for something sweet on a validated 100-point VAS accessed via a PC or a tablet (30, 31). These measures will be completed on an electronic Questionnaire Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be measured using a culturally adapted version of the Leeds Food Preference Questionnaire (LFPQ)(32) on a computer desktop. Appetite sensations measured by VAS will be repeated after the LFPQ and before the researcher brings the blinded intervention product served with 200 mL of water. The participant will be instructed to take one bite, then answer questions regarding sensory-specific satiety and expected satiety by VAS (33, 34). The participant will be asked to consume the rest of the product over a period of 5-10 minutes, depending on the time required to consume the matrix and asked to complete a set of appetite sensation questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture peak PP response (35) (yoghurt will be consumed faster than other products therefore blood samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30, 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In between measurements, participants will remain seated in a quiet area, free from food-related sensory stimuli and read/listen to music/use a computer (provided there is no material with reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete the participant will be offered water or a snack before leaving the laboratory. Participants will be reminded about the consumption of the products at home and that they will receive a phone call the next day to complete a 24-hour diet recall and report any GI symptoms. Following the end of the trial, participants will be debriefed if requested and offered the chance to complete a survey about the conduct of the study.

[Figure 2]

Intervention products

There will be 1 control product (sucrose-containing manufactured products) and 2 no added/reduced sugar reformulated products based on the same food matrix - including 2 modulations of S&SE content (inclusion as individual S&SE or S&SE blends). The reformulated products have a target of ≥30% reduction in energy and/or sugar to achieve the status of 'reduced sugar' by EU regulation No 1047/2012. This will not be possible in all products, therefore 'no added sugar' will be applied to products who do not meet the criteria

(biscuits and cakes). The control products will range from 305-360 kcal (1286-1516 kJ), while the intervention products will range from 242-326 kcal (1013-1368 kJ) (full product ingredients in **Supplemental Material 2**). Intervention and control products will be matched for sweetness intensity, flavour and physical appearance.

The 2 individual S&SEs tested are Neotame and Stevia Rebaudioside M and the 2 S&SE blends are Sucralose/Acesulfame K blend and Mogroside V/Stevia Rebaudioside M blend.

Data Collection and Outcomes

Table 1 Details at which time point(s) data are collected at the CID.

Table 1: Data Collection and Timepoints for each CID

		Baseline or 0' (fasting)	10'	15'	20'	30'	45'	60'	120'	180'	Next day
Primary endpoint	Subjective appetite (VAS for hunger, desire to eat, fullness, prospective food consumption)	X	X		X	X	X	X	X	X	
Behavioural endpoints	Food preference & reward (LFPQ)	X			X						
	Food cravings (CoEQ)	X									
	Energy intake (24-hour dietary recall)										X
	Expected satiety	X (1 bite)									
	Sensory-specific satiety	X (1 bite)	X								
	Other appetite ratings (e.g., thirst, nausea, bloating. appetite for something sweet/savoury)	X	X		X		X	X	X	X	
Metabolic endpoints	Glucose and insulin	X	X	X		X		X	X		
	Pancreatic polypeptide (PP)*	X	X	X		X					
	GLP-1 and ghrelin	X				X		X			
	Lipaemia (triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol)	X				X		X	X		
Health endpoints	Liver function (ALT, AST, GGT, FL index, TyG index)	X							X		
	HbA1c	CID1 & 6									
	24-hour GI side effects (self-report)										X

* Timepoints for PP are earlier for yoghurt study

Abbreviations: ALT - alanine transaminase, AST – aspartate transaminase, GGT - gamma-glutamyltransferase, FL index - fatty liver index, TyG index - triglycerides and glucose index VAS - visual analogue scale, LFPQ - Leeds Food Preference Questionnaire, CoEQ - Control of Eating questionnaire, GLP-1- Glucagon-like peptide 1, HDL - High density lipoprotein, LDL - Low density lipoprotein, HbA1c - Haemoglobin A1c, GI - Gastrointestinal, CID - Clinical Investigation Day.

Primary Outcome

This trial has one primary outcome which is the iAUC for the 180-minute composite appetite score based on hunger, fullness (reverse scored), desire to eat and prospective food consumption (36). These subjective appetite ratings will be measured throughout the CIDs using VAS on the QDP. The trapezoid method will be used for the calculation of iAUC (37).

Secondary Outcomes

Food preference and reward

Food preference and food reward will be measured at all CIDs using the LFPQ (32). Changes will be determined by comparing the relative preference/food choice, explicit liking and implicit wanting for high-fat sweet, low-fat sweet, high-fat savoury and low-fat savoury foods, and fat/sweet appeal bias scores in the fed and hungry states between the reformulated and control products.

Food cravings

Food cravings will be determined at all CIDs by craving control, craving for sweet and savoury scores from the CoEQ (29), which is a 21-item questionnaire with responses recorded on a 100-point VAS (1 item allows for text response).

Energy intake

Energy intake will be measured by a 24-hour dietary recall (using the multiple pass method (38)), which will be conducted by a trained dietitian or research staff over the telephone. Participants will be asked to recall all food and drink consumed during the 24-hour period since leaving the laboratory. Participants will receive training on reporting food portions using the Australian Health Survey Food Model Booklet (39) or similar culturally adapted resources.

Compensatory eating behaviour will be determined from the analysis of the 24-hour dietary interview data using energy intakes calculated with national nutritional software. The following variables will be considered: 1) Energy and macronutrient distribution and 2) Percent energy compensation (%EC), defined as the adjustment of energy intake (EI) provoked by the intervention products (40), (see **Supplemental Material 3** for further information).

Expected satiety and sensory specific satiety

Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (33, 41) and sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire (34) after one bite and full consumption (10') of the product. Responses to both questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).

Other behavioural ratings

Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).

Biochemical measures

Blood for plasma analyses will be centrifuged at 1500 *g* at 4°C for 10 minutes immediately after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately after collection. Plasma and serum aliquots will be stored at -80°C until shipment for analyses to Bioaitriki Laboratories (central lab) in Athens, Greece.

Insulin concentrations will be determined by chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system. Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay (Abbott) which consists of two separate concentration measurements: glycated haemoglobin (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-cholesterol will be determined by an accelerator selective detergent method (Ultra HDL assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye formation method (Direct LDL assay, Abbott). AST and ALT will be determined by enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated according to information provided in **Supplemental Material 5**.

Gastrointestinal (GI) side effects

Any reported unusual GI side effects, including abdominal pain/cramps, heartburn, stomach acid/reflux, nausea, vomiting, abdominal rumbling, bloating, belching, excess gas/wind, bowel movements, stool type, etc. during the study will be recorded at the phone call the day after each CID and each day during the at-home intervention in a booklet including the Bristol Stool Form Scale (42). The GI symptoms check has been based on the validated Gastrointestinal Symptoms Rating Scale (GSRS) tool (43).

STATISTICAL ANALYSIS PLAN

Per protocol analysis will include participants that completed all 6 CIDs and had a level of adherence to the product consumption >80%. The main evaluations for this trial will be to investigate differences between the intervention products (2 no added/reduced sugar reformulated S&SE products and 1 sucrose-sweetened control). Where this is not appropriate for some of the secondary outcomes, descriptive analyses will be used to interpret differences. Data will be pooled across the split-site (Leeds and Lyon) study using the biscuit matrix. Data will be presented as means and standard deviation. Outcome variables will be checked for normality and transformed where necessary. Analyses will include repeated measures ANOVA or mixed-effects regression models to compare S&SE product conditions versus control in a within-subjects design. Covariates (age, sex, BMI, fasting appetite levels, intervention site, treatment order, adverse events and medication changes) will be adjusted for where necessary (e.g., if they correlate with other variables). Pearson's correlations will be used to explore relationships between variables. Adjustment for multiple comparisons will be applied where necessary. The American Statistical Association's policy statement on p-values (44) advises that all p-values from specified statistical models be reported along with point estimates, effect size and confidence intervals to help interpret the compatibility of the data with the study outcomes, therefore this procedure will be followed. Otherwise, the level of significance will be set at 0.05.

Safety analysis

Information relating to adverse events (including events relating to GI side effects) and concomitant medication will be tabulated and summarised descriptively.

ETHICS AND MONITORING

Each intervention site will obtain ethical approval from their local ethical committee. All study procedures will be conducted in accordance with the Helsinki Declaration and the study protocol has been registered in a public database (clinicaltrials.gov NTC04633681; **Supplemental Material 6**). To the extent relevant and reasonable International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines will be used, and standard operating procedures (SOP) will be developed to facilitate the same performance and compliance with the protocol in each centre. All personal data is handled confidentially and stored in accordance with applicable law, GDPR and local laws (see Appendices). All participants will receive written and oral information about the study and only trained study personnel will provide information, monitor and attest signing of the informed consent form. Where required, monitoring of intervention sites will be performed during the study by the University of Navarra depending on local regulations.

TRIAL STATUS

Recruitment opened in May 2021for the trial at the Leeds and Lyon intervention sites using the biscuit matrix, with last participant last visit expected by June 2022. Recruitment for the trial at Lyon using the cake matrix opened in February 2022. The trials at Liverpool, Copenhagen and Pamplona will start recruiting in Spring 2022.

PUBLICATION

After completion of the study, the findings will be submitted for publication in an international peer-reviewed scientific open access journal and other relevant media. Research data from the trial will be deposited in an open access online research data archive (for example Zenodo).

FUNDING AND COMPETING INTERESTS STATEMENT

The present study is funded by the Horizon 2020 program: Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability (acronym: SWEET, grant no: 774293). The current study was initiated by Prof. G. Finlayson as part of the Work Package 2 of the SWEET project. The study receives funding from the Horizon2020 program (9 million Euros) to cover salary for project personnel, supplies, remuneration and dissemination of results. The amount is deposited in a project account subject to public revision. The funder has no role in the study design, interpretation of data or publication of material.

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AUTHORS' CONTRIBUTIONS

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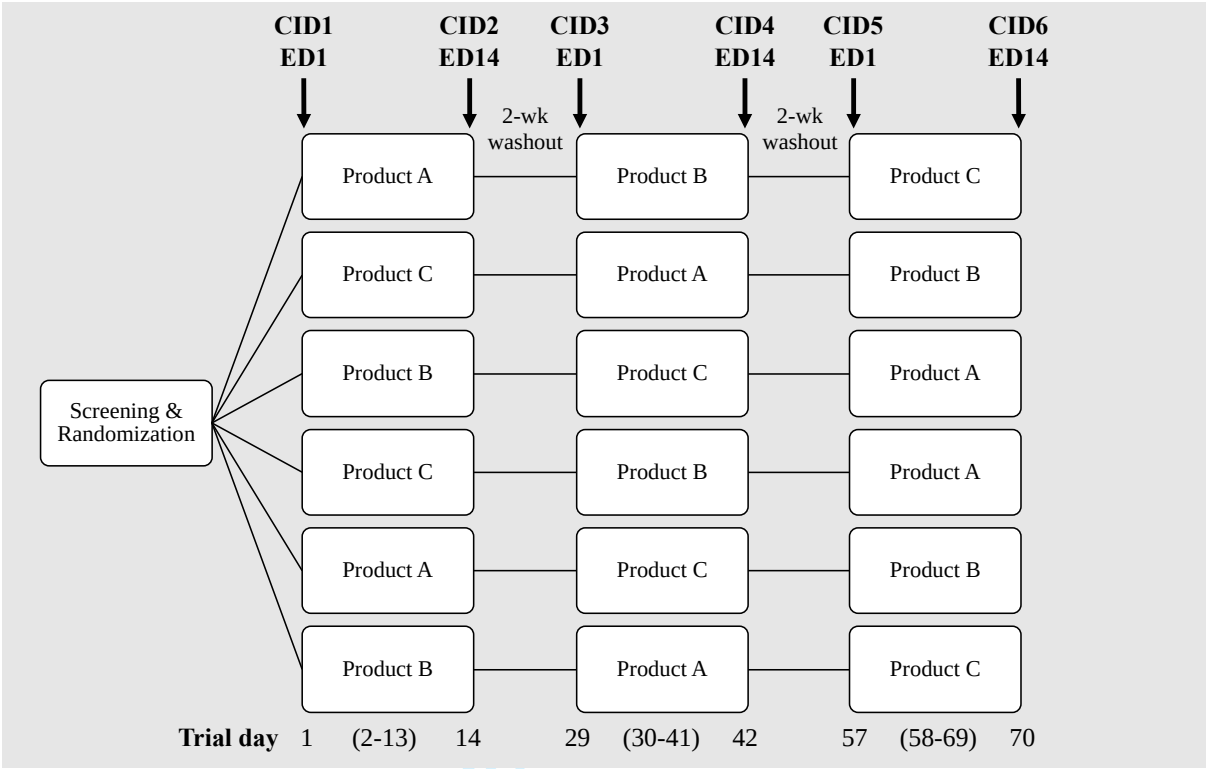

Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to order one will consume product A in the lab on clinical investigation day (CID) 1/exposure day (ED) 1 and then every day at home until CID2/ED14 when it is consumed in the lab again. After a 2-week washout, the participant returns to the lab and repeats the study block with product B, followed by another 2-week washout, followed by the final study block with product C.

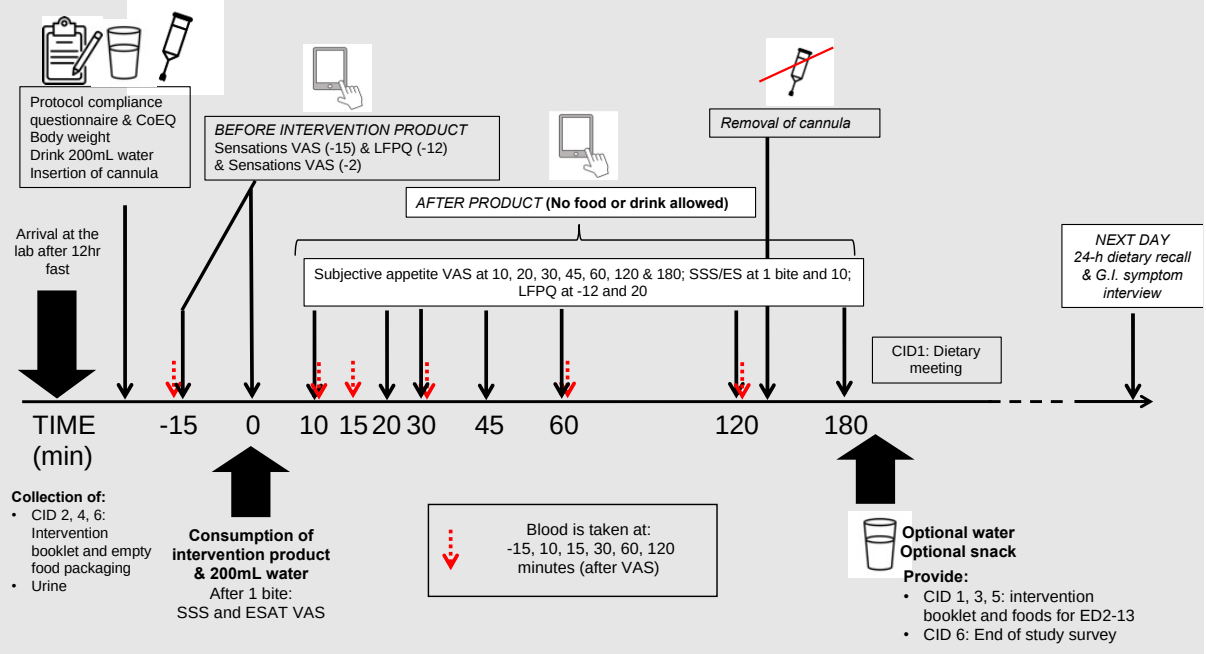

Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix. CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.

Supplemental Material 1: Exclusion Criteria

General Criteria

- Blood donation < 3 month prior to study or for full duration of the study.
- Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g., veganism) or history of anaphylactic reaction to any food.
- Likelihood for disordered eating defined as a score ≥20 on the EAT-26 test
- Currently dieting to lose weight.
- Having lost or gained >4.5 kg in the last 3 months.
- Smoking or having quit <3 months prior to study.
- Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months.
- Performing >10 h of intense physical activity per week in the last 3 months.
- Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift.
- Self-reported use of drugs of abuse within the previous 12 months.
- For women: Pregnancy, lactation.
- Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study).
- Insufficient communication in the national language.
- Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen.
- Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician.
- Simultaneous participation in other relevant clinical intervention studies.
- Previous university or college training related to eating behaviour research.

Medical conditions as known by the person

- Self-reported eating disorders.
- Diagnosed anaemia.
- Diagnosed diabetes mellitus.
- Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer.
- Active inflammatory bowel disease, celiac disease, chronic pancreatitis, or other disorder potentially causing malabsorption.
- History of G.I. surgery with permanent effect (i.e., surgical treatment of obesity).
- Medical history of CVD (e.g., current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease).
- Significant liver disease, e.g., cirrhosis (fatty liver disease allowed).
- Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed).
- Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3 months.
- Psychiatric illness (e.g., major depression, bipolar disorders).

Medication

- Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety, or body weight incl. food supplements.

Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months.

- Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months).

Supplemental Material 2: Product Ingredients List

Table 1: Proposed energy and nutrient composition of the intervention products

	Control		Reformulation	
Cake with fruit filling	Per 100g	Per portion (85g/ 3 cakes)	Per 100g	Per portion (85g/ 3 cakes)
Energy (kcal)	391	332	343	292
Energy (kJ)	1638	1392	1427	1213
Fats (g)	16.6	14.1	16.5	14.0
Sat. fats (g)	1.7	1.4	1.7	1.4
Carbs (g)	56.8	48.2	57.0	48.4
Sugars (g)	28.3	24.1	1.3	1.1
Polyols (g)	3.7	3.1	28.4	24.1
Fibre (g)	1.4	1.2	1.3	1.1
Proteins (g)	5.7	4.8	5.7	4.8
Salt (mg)	0.4	0.3	0.4	0.3
Biscuit	Per 100g	Per portion (3 biscuits)	Per 100g	Per portion (3 biscuits)
Energy (kcal)	423	360	384	326
Energy (kJ)	1783	1516	1609	1368
Fats (g)	11.2	9.5	11.5	9.8
Sat. fats (g)	7.11	6.0	7.33	6.2
Carbs (g)	75.9	64.5	76.2	64.8
Sugars (g)	24.7	21.0	1.8	1.5
Polyols (g)	3.7	3.1	22.7	19.3
Fibre (g)	0.7	0.6	2.4	2.0
Proteins (g)	6.5	5.5	6.6	5.6
Salt (mg)	0.7	0.6	0.7	0.6
Creamy yoghurt	Per 100g	Per portion (135g 1 serving)	Per 100g	Per portion (135g 1 serving)
Energy (kcal)	226	305	180	242
Energy (kJ)	943	1286	750	1013
Fats (g)	18.5	25.0	16.3	22.0
Sat. fats (g)	8.3	18.7	8.3	18.7
Carbs (g)	8.12	10.96	1.71	2.31
Sugars (g)	8.12	10.96	1.71	2.31
Polyols (g)	0.00	0.00	0.00	0.00
Fibre (g)	0.00	0.00	0.00	0.00
Proteins (g)	6.50	8.78	6.50	8.78
Salt (mg)	0.14	0.32	0.14	0.32
Chocolate	Per 100g	Per portion (60g 1 bar)	Per 100g	Per portion (60g 1 bar)
Energy (kcal)	500	325	477	310
Energy (kJ)	2098	1364	2004	1303
Fats (g)	31	20	31	20
Sat. fats (g)	18	12	18	12
Carbs (g)	46	30	46	30
Sugars (g)	45	29	31	20
Polyols (g)	0	0	12	8
Fibre (g)	8	5	8	5
Proteins (g)	5	3	5	3
Salt (mg)	5	3	8	5
Honey ball breakfast cereal	Per 100g	Per portion (60 g of cereals + 125 ml (121 g) of milk)	Per 100g	Per portion 60 g of cereals + 125 ml (121 g) of milk)
Energy (kcal)	173	320	153	283
Energy (kJ)	731.2	1353.8	641.7	1187.2
Fats (g)	3.26	6.03	3.16	5.84
Sat. fats (g)	1.64	3.04	1.63	3.01
Carbs (g)	31.17	57.66	25.59	47.33

Sugars (g)	12.03	22.26	5.02	9.28
Polyols (g)	0	0	4.86	9.00
Fibre (g)	0.86	1.60	6.64	12.28
Proteins (g)	4.38	8.11	4.21	7.79
Salt (mg)	0.35	0.64	0.33	0.60

Supplementary Material 3 – Energy Intake Calculation

Percent energy compensation (%EC) was derived from the dietary recall data as previously reported by Zandstra et al.[1], and Almiron-Roig et al[2]. Briefly, %EC was calculated as:

$$\%EC = [\ (EI_{\text{Control Product}} - EI_{\text{Reformulated Product}})/|EP|]*100$$

where EI represents the cumulative energy intake 24-h post consumption under the control product or under the reformulated product conditions, excluding the energy of the product itself. EP (as an absolute value) represents the difference in energy between the full-energy-containing preload (i.e., control product) and the lower-energy-containing preload (i.e., reformulated products). For example, if the control product has a value of 325 kcal and the reformulated product has a value of 250 kcal, then EP=325-250 or 75 kcal).

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2. Almiron-Roig, E., Palla, L., Guest, K., Ricchiutu, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews, 71*(7), 458-473. https//doi.org/10.1111.nure.12048

Supplemental Material 4: Questions used to assess product taste text, subjective appetite sensations, sensory specific satiety and expected satiety

Food Taste Test (FTT) (Conducted at screening)

Screen 1

You will now be presented with a food that we will ask you to evaluate. Please follow the instructions as they appear on the screen.

Screen 2

1. Take a mouthful of the food provided.
2. Chew while counting to 5.
3. Swallow.
4. Answer the question by moving the arrow to the left or to the right.

How **pleasant was this food**?

Not at all pleasant — Extremely pleasant

Screen 3

Thank you. This is the end of the taste test.

Please call the investigator after submitting your answer.

Subjective Appetite Questions (used during all CIDs)

Considering how you feel **right now**, give your answer to each of the following questions by moving the arrow to the left or to the right at the point that best represents your experience. The list below is the complete list of questions used via visual analogue scales.

1. How **hungry** do you feel?
2. How **full** do you feel?
3. How **thirsty** do you feel?
4. How strong is your **desire to eat**?
5. How **much** do you think you could **eat** right now?
6. How **nauseous** do you feel?
7. How **bloated** do you feel?
8. How strong is your appetite for something **savoury**?
9. How strong is your appetite for something **sweet?**

Sensory Specific Satiety Questionnaire (assessed after 1 bite and after consumption of product)

After 1 bite:
Please take a bite of the food and keep the food in your mouth while rating the food. Swallow the food only when your rating is complete.

How **pleasant** is the taste of the food right now?

At 10 minutes:
How **pleasant** is the taste of the food now that you have finished eating it?

Expected Satiety (ESAT) (assessed after 1 bite and after consumption of product)[1]

After 1 bite:
After having taken 1 bite of the food and looking at the whole food portion, how much will this portion of food stop you from feeling hungry between meals?

At 10 minutes after full consumption and after SSS rating:
How much will this food stop you from feeling hungry between meals?

Supplemental Material 5: Fatty liver index and triglyceride glucose index calculation

Calculation of Fatty Liver Index:

Some of the blood parameters will be used to calculate a Fatty Liver index (FL) using the formula of Bedogni et al [1],with measured values for BMI, fasting TG (mg/dL), fasting GGT (U/L) and waist circumference (cm), as follows:

$$FLI = (e^{0.953*\log e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e(\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) / (1 + e^{0.953*\log e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e(\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) * 100$$

Calculation of Triglyceride Glucose Index:

The formula of Simental-Mendía et al.[2] will be measured with measured fasting TG (mg/dL) and fasting glucose (mg/dL), by dividing the Ln of the TG *glucose product by 2:

TyG index = Ln [(fasting triglycerides) (mg/dL) x fasting glucose (mg/dL)] / 2

1. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterology*, *6*(1), 33. https://doi.org/10.1186/1471-230X-6-33

2. Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, *6*(4), 299–304. https://doi.org/10.1089/met.2008.0034

Protocol for acute and repeated dose impact of sweeteners and sweetness enhancers on appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-over, randomised, controlled trial in people with overweight/obesity. The SWEET project.

Supplemental Material 6: Trial Registration Data Set

Trial registration data	
Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT04633681
Date of registration in primary registry	November 2021
Secondary identifying numbers	N/A
Source(s) of monetary or material support	European Union Horizon 2020 Program
Primary sponsor	European Union Horizon 2020 Program
Secondary sponsor(s)	N/A
Contact for public queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Contact for scientific queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Public title	Impact of Sweeteners on Behaviour, Physiology & Health (SWEET-WP2-P2)
Scientific title	Acute and Repeated Impact of Sweeteners and Sweetness Enhancers on Food Behaviour, Physiology & Health (SWEET Work Package 2 Phase 2)
Countries of recruitment	Denmark, France, Spain, United Kingdom
Health condition(s) or problem(s) studied	Eating Behaviour
Intervention(s)	Consumption of food product with sweetener and sweetness enhancer Consumption of sucrose-sweetened control food product
Key inclusion and exclusion criteria	Ages eligible for study: ≥ 18 ≤60 years ; Sexes eligible for study :both; Accepts healthy volunteers: yes
	Inclusion criteria: BMI 25-35kgm2; Use of contraceptive methods or not planning to become pregnant for the duration of the study (women only); Regular consumption of sugar-containing foods and willing to consume sugar and artificially-sweetened food products; Liking of the intervention foods defined by a response of 'Yes' for the product during the pre-screening interview and a score of 40% or above on the Liking Visual Analogue Scale for the sucrose-sweetened control product; Able to participate on the Clinical Investigation Days during normal working hours; Healthy as determined from the self-reported medical history or when a clinical condition exists, when this is considered to be irrelevant (i.e. not influencing study outcomes) for the study by the study medical doctor; Consuming breakfast regularly (at least 5 days per week); Able to understand and be willing to sign the informed consent form, and to follow all the study procedures and requirements; Capacity to store at-home intervention quantity of intervention product
	Exclusion criteria: Blood donation < 3 month prior to study or for full duration of the study; Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g. veganism) or history of anaphylactic reaction to any food; Likelihood for disordered eating defined as a score ≥20 on the Eating Attitudes Test; Currently dieting to lose weight; Having lost or gained >4.5 kg in the last 3 months; Smoking or having quit <3 months prior to study; Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months; Performing >10 h of intense physical activity per week in the last 3 months; Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift; Self-reported use of drugs of abuse within the previous 12 months; Pregnancy, lactation (women only); Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study); Insufficient communication in the national language; Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen; Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician; Simultaneous participation in other relevant clinical intervention studies; Previous university or college training related to eating behaviour research; Self-reported eating disorders; Diagnosed anaemia; Diagnosed diabetes mellitus; Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer; Active inflammatory bowel disease, coeliac disease, chronic pancreatitis or other disorder potentially causing malabsorption; History of G.I. surgery with permanent effect (i.e. surgical treatment of obesity); Medical history of Cardiovascular Disease (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease); Significant liver disease, e.g. cirrhosis (fatty liver disease allowed); Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed); Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3months; Psychiatric illness (e.g. major depression, bipolar disorders); Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety or body weight incl. food supplements. Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for

	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering medication, if the dose has changed during the last3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months)
Study type	Interventional Allocation: Randomised. Double-blind, within-subjects, cross-over trial
	Primary purpose: Evaluation Phase: N/A
Date of first enrolment	April 2021
Target sample size	213
Recruitment status	Recruiting
Primary outcome(s)	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
Key secondary outcomes	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers: Glucagon-like peptide-1 (GLP-1)Incremental area under the curve for blood GLP-1 concentrations in response to each product (120 min post intake);Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product (120 min post intake).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	12
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supplemental Material 6
Protocol version	#3	Date and version identifier	6
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	15
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7 and 15
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8-9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A: No safety/critical efficacy issues present

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15; Supplemental Material 4
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Data from participants who discontinue/ deviate from protocol not collected
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15; appendices
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A, no analysis included
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A, no analysis included

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A: No safety/critical efficacy issues present
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A: No safety/critical efficacy issues present
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A: No safety/critical efficacy issues present
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Appendices
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A: No safety/critical efficacy issues present
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendices

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12

BMJ Open

Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with overweight/obesity: The SWEET Project

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Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with overweight/obesity: The SWEET Project

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Abstract

Introduction: Intake of free sugars in European countries is high and attempts to reduce sugar intake have been mostly ineffective. Non-nutritive sweeteners and sweetness enhancers (S&SEs) can maintain sweet taste in the absence of energy, but little is known about the impact of acute and repeated consumption of S&SE in foods these products on appetite. This study aims to evaluate the effect of acute and repeated consumption of 2 individual S&SEs and 2 S&SE blends in semi-solid and solid foods on appetite and related behavioural, metabolic and health outcomes.

Methods and Analysis: A work package of the SWEET project, this study consists of 5 double-blind randomised cross-over trials which will be carried out at 5 sites across 4 European countries, aiming to have n=213. Five food matrices will be tested across 3 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends and no added sugar). Participants (body mass index (BMI) 25-35 kg/m^2; aged 18-60 years) will consume each formulation for 14 days. The primary endpoint is composite appetite score (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour postprandial incremental area under the curve during clinical investigation days on day 1 and 14.

Ethics and dissemination: The trial will be approved by national ethical committees before starting recruitment and will be conducted in accordance with the Declaration of Helsinki. Results will be published in international peer-reviewed open access scientific journals. Research data from the trial will be deposited in an open access online research data archive.

Trial registration number: Clinicaltrials.gov: NCT04633681

Strengths and Limitations of this study:

- The trial is the first of its kind to investigate the effects of acute and repeated exposure to 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary (chocolate) and breakfast cereal.
- This trial includes a large range of outcomes across behaviour, physiology, and health from persons living in Northern, Central and Southern Europe.
- The COVID-19 pandemic resulted in changes to the design of the studies in the trial. Originally, all products were to be tested across 2 sites, but the reduced time frame means this is not possible for some products.

- Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will be reported descriptively in these 2 studies where appropriate.

BACKGROUND AND RATIONALE

The global increase in the prevalence of obesity and its associated diseases is driven by a range of internal factors, involving genetic, behavioural, and metabolic determinants along with permissive external factors from the physical, social, and public (nutritional) policy environment (1). One of the main behavioural drivers involves a diet too rich in energy intake relative to energy expenditure. Free sugar intake (derived from sugar added to foods and beverages by the manufacturer or consumer) is one nutritional component that has gained focus because of its low nutritional value (lack of vitamins, minerals or fibre) and its potential to add to overall energy consumed, facilitating weight gain (2), and potential altered appetite and endocrine responses to carbohydrates (sugars) relative to other macronutrients (3).

Simply restricting free sugars from the diet without substitution may reduce diet palatability or contribute to changes in sweet craving (4), particularly in women (5), resulting in poor acceptance.. The replacement of free sugars with non-nutritive sweeteners and sweetness enhancers (S&SEs) in food products is one method to reduce sugar intake while maintaining acceptance and palatability of the diet. S&SEs have increasingly been employed over recent years to reduce the energy and sugar content of foods; however, their impact on appetite- and health-related outcomes is somewhat unclear (6).

The effect of S&SEs on appetite is difficult to summarise due the types of studies, comparisons and S&SEs being used. One of the reasons for the current partial understanding of the appetitive and metabolic effects of S&SEs in humans is that different S&SEs are commonly assumed to have similar behavioural effects (6-8). Only recently, one 12-week investigation of 4 distinct S&SEs reported directionally dissimilar effects of saccharin compared to sucralose on body weight (9). A recent review comparing different S&SEs suggests that some have the potential to enhance appetite, but these effects do not follow through to subsequent energy intake (6). A recent meta-analysis detailed the impact of no- or low-energy sweetened preloads compared to conventionally sweetened preloads on *ad libitum* energy intake. They concluded that similar effects on energy intake were seen due to only partial compensation being evident (although total energy intake was lower in the no- or low-energy sweetener) (10). Furthermore, recent studies have highlighted that S&SEs may

reduce sweet food cravings and therefore reduce sugar intake (11) appetite and energy intake (12). Overall, there is currently insufficient evidence to make a clear conclusion about the effect of S&SEs on appetite and energy intake. Indeed, the 11 S&SEs that are currently approved for use in the EU are chemically heterogeneous and absorbed, metabolised, and excreted differently (13). Furthermore, most investigations of the relationship between S&SE intake and health outcomes have used beverages as the vehicle (14) ; these have recently been reviewed (15). Since the amount of S&SEs in the food supply is increasing in response to consumer demand (16) and policy (e.g., 'sugar taxes'(17, 18); EU initiatives (19, 20)), there is a pressing need to examine the appetite-related behavioural and metabolic consequences of consuming S&SEs particularly in semi-solid and solid food matrices. Furthermore, it should be acknowledged that differences between acute and longer-term effects of S&SEs may not be the same (21) and this needs investigating further.

AIMS AND OBJECTIVES

The main objective of this study is to evaluate the acute (1-day) and repeated (14-day) effects of 2 individual S&SEs and 2 S&SE blends in reformulated reduced or no added sugar food products (using 2 modulations of S&SEs per matrix) on appetite and related behavioural, metabolic and health outcomes in adult men and women with overweight or obesity.

The hypotheses are:

H_1 Consumption of no added/reduced sugar products reformulated with S&SEs will result in an altered incremental area under the curve (iAUC) appetite score, compared with the sucrose-sweetened control product after repeated compared with acute consumption.

H_2 There will be differences between the no added/reduced sugar and sucrose-sweetened formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-related (liver function and gastrointestinal (GI) side effects) outcomes.

TRIAL DESIGN

This study is part of the wider SWEET project (https://sweetproject.eu/) funded by the European Commission Horizon 2020 program. It is a multicentre double-blind, randomised

cross-over trial conducted across 5 intervention sites in 4 countries, with 3 product formulations (sucrose-sweetened control vs 2 individual S&SEs or S&SE blends) over 5 intervention product types (cake, biscuits, yoghurt, chocolate, and breakfast cereal matrices) aiming for a total of 213 completers. The protocol is reported as per the SPIRIT reporting guidelines (22). While this study addresses the short term impact of specific S&SEs vs added sucrose on appetite, another work package in the SWEET project will examine the long term (1-year) impact of a weight loss and weight maintenance intervention with or without S&SE as part of a healthy diet (bmjopen-2022-061075).

Sample Size Determination

The following calculations apply to the studies involving biscuit, yoghurt and chocolate matrices:

Primary outcome: Power calculations showed that to detect a minimum difference of 8 mm in appetite ratings on a 100 mm visual analogue scale (VAS) with 80% power, alpha 0.05, and based on a published within-subject SD of 14.4 mm in VAS measures (23), an overall sample of 53 completers (both sexes, same BMI group, across all centres) would be needed (24; p.30) per matrix. We expect this sample size will be sufficient to detect iAUC differences in the appetite response relative to control of around 8–10%, considered to be of practical relevance (25).

Secondary outcomes: Due to the number of secondary outcomes in this study, it was not feasible to conduct power calculations for all outcomes. However, we consulted published studies (e.g. Yeomans et al, 2016 (24)) which utilized a similar design and demonstrated effects of small nutritional manipulations on various gut peptides. In these studies, sample sizes ranged from 12-23 participants, giving us confidence that a sample of 53 participants per matrix should be sufficient to detect differences with clinical significance.

Due to the impact of the COVID-19 pandemic on the trial (detailed later), the cake and breakfast cereal studies were scaled down according to reduced feasibility at each intervention centre to n=24 (cake) and n=30 (breakfast cereal), and no blood samples will be collected in the cake study. The primary outcome will be reported descriptively in these 2 studies where appropriate and reflected in the study registration and protocol.

STUDY SETTING

This trial is conducted across 5 intervention sites in 4 countries across 3 regions of Europe, with each site testing a different product, whilst following the same protocol. Western Europe: Leeds (University of Leeds, UK) will test biscuits; Liverpool (University of Liverpool, UK) will test chocolate; Lyon (Centre de Recherche en Nutrition Humaine Rhône Alpes, France) will test biscuits and cakes. Northern Europe: Copenhagen (University of Copenhagen, Denmark) will test cereal. Southern Europe: Pamplona (University of Navarra, Spain) will test yoghurt. University's of Leeds and Navarra are the leaders of this work package, whilst University of Liverpool is the co-ordinating centre of the SWEET project in its entirety.

PATIENT AND PUBLIC INVOLVEMENT

During the study, research staff discuss with participants about their experiences of the clinical investigation days, examinations, participant information and written materials, etc. with the aim to understand and improve participants' experiences in current and future studies of this nature. This is also captured in an end of study survey.

ELIGIBILITY CRITERIA

Male and female adults aged 18-60 years, with a BMI 25-35 kg/m^2 are eligible. Participants are required to regularly consume sugar-containing foods and willing to consume sugar and sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-26) (26) score <20 and a short sweet food frequency questionnaire score ≥3 of 11, in addition to rating the control product as ≥40% on a 100-point liking VAS during the taste test and be willing to consume the product during the duration of the trial. All exclusion criteria are listed in **Supplemental Material 1**.

INTERVENTION

Each trial will begin with an initial exposure to one of the 3 assigned product formulations under controlled laboratory conditions (clinical investigation day, CIDs 1, 3, 5 – exposure day 1), followed by repeated daily consumption of the same product at home for 12 (± 2 days) and a final exposure in the laboratory on day 14 (± 2 days) under identical conditions as the first exposure (CIDs 2, 4, 6 – exposure day 14), resulting in all participants completing the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be followed by a wash out period of 14-21 days between formulations. During the at-home periods, participants will consume a portion of the product at a time and place they choose

using a substitution strategy for similar energy/sweetness foods in their habitual diet. Foods habitually consumed of an equivalent energy density/sweetness are identified using participant's answers to a food frequency questionnaire and an energy equivalent guide, with a decision making tree developed to identify the most suitable foods to substitute for each intervention product. This strategy is supported by advice and agreement from the research officer/dietitian. Compliance will be monitored by an intervention booklet completed daily and by return of empty food packaging. All food products are provided in the same blinded container/wrapping. The study duration for each participant will be a minimum of 70 days (plus 7-14 days allowance for extended washout to aid scheduling of CIDs).

Recruitment and Screening

Participants will be recruited via a variety of routes e.g., study databases, webpages, social media, posters, and flyers. Potential participants will be pre-screened using an online or telephonic pre-screening questionnaire in accordance with the inclusion and exclusion criteria. Candidates passing pre-screening will be invited to attend an information session, either online or in-person, where they will be given detailed information about the study and invited to participate in a Q&A session. Candidates who wish to participate in the study will provide written informed consent and sign a general data protection regulation (GDPR) form before being fully screened. The screening session will be performed in-person or online, and will consist of anthropometric measurements (height, weight, waist and hip circumference; all confirmed in-person at CID1 for participants being screened online); eligibility questionnaires (EAT-26 (26) and short sweet food frequency questionnaire); baseline questionnaires (A socio-demographic questionnaire, a questionnaire to assess habitual sweet food consumption, including regular and S&SE sweet foods (SWITCH sweet food frequency questionnaire (SWFFQ)) (27), a questionnaire to assess habitual physical activity (International Physical Activity Questionnaire (IPAQ)) (28) and a consumer perspective questionnaire); an eligibility taste test of the control intervention product here participants rated the pleasantness of the product on a 100mm VAS after taking one bite and chewing for 5 seconds (a score of >40mm was required for inclusion into the study). Candidates who pass the screening session will be enrolled into the study.

Randomisation and blinding

A Latin square design (6 treatment orders) will be used to randomly allocate product sequence into blocks of 6, as shown in Figure 1. The person responsible for generating the

sequences for all sites will not have any study related tasks e.g., inclusion or examination of participants. Each sequence will be stratified by sex (female/male) and age group (18-45 years/46-60 years). When feasible, a female/male ratio of minimum 60/40 was also considered to reflect the target population characteristics.

Blinding of the intervention products (reformulated and control products) will be done by the manufacturers. As such, blinding of the research and central laboratory staff will take place allowing for a double-blind intervention. Moreover, the statistical analyses of the main outcome variable will be done without breaking the intervention product-assignment code before the analyses are finalised.

[Figure 1]

Clinical investigation days

Prior to each CID, participants will be asked to consume a similar evening meal at the same time, before fasting for a minimum of 12 hours and a maximum of 15 hours. High intensity physical activity, alcohol, and coffee will not be allowed for 12 hours before arriving to the laboratory. Two glasses, approximately 500 mL, of non-carbonated water will be allowed during the fasting period. Participants will provide a spot urine sample collected max 24 hours before each CID and will be analysed for the presence of specific S&SEs.

The CID procedures are outlined in Figure 2. CID start times will be scheduled in the morning between 8.00 am and 10.30 am and participants will start all 6 CIDs at the same time. Participants will complete a protocol compliance questionnaire to verify the above requirements regarding diet, physical activity, etc. If compliance has been breached, staff will reschedule the CID (within the maximum 14 days allowed, otherwise a protocol deviation will be recorded). If compliance has been achieved, participants will then fill in the Control of Eating Questionnaire (CoEQ)(29) to assess cravings over the last 7 days, followed by a body weight measurement. Participants will consume 200 mL of water before having an intravenous cannula inserted into an antecubital vein by qualified personnel. A baseline fasting blood sample will be taken 15 minutes after insertion of the cannula. Once the fasting sample has been taken, participants will complete fasting subjective appetite ratings for hunger, fullness, thirst, desire to eat, prospective intake, nausea, bloating, appetite for

something savoury and for something sweet on a validated 100-point VAS accessed via a PC or a tablet (30, 31). These measures will be completed on an electronic Questionnaire Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be measured using a culturally adapted version of the Leeds Food Preference Questionnaire (LFPQ)(32) on a computer desktop. Appetite sensations measured by VAS will be repeated after the LFPQ and before the researcher brings the blinded intervention product served with 200 mL of water. The participant will be instructed to take one bite, then answer questions regarding sensory-specific satiety and expected satiety by VAS (33, 34). The participant will be asked to consume the rest of the product over a period of 5-10 minutes, depending on the time required to consume the matrix and asked to complete a set of appetite sensation questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture peak PP response (35) (yoghurt will be consumed faster than other products therefore blood samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30, 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In between measurements, participants will remain seated in a quiet area, free from food-related sensory stimuli and read/listen to music/use a computer (provided there is no material with reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete the participant will be offered water or a snack before leaving the laboratory. Participants will be reminded about the consumption of the products at home and that they will receive a phone call the next day to complete a 24-hour diet recall and report any GI symptoms. Following the end of the trial, participants will be debriefed if requested and offered the chance to complete a survey about the conduct of the study.

[Figure 2]

Intervention products

There will be 1 control product (sucrose-containing manufactured products) and 2 no added/reduced sugar reformulated products based on the same food matrix - including 2 modulations of S&SE content (inclusion as individual S&SE or S&SE blends). The reformulated products have a target of ≥30% reduction in energy and/or sugar to achieve the status of 'reduced sugar' by EU regulation No 1047/2012. This will not be possible in all products, therefore 'no added sugar' will be applied to products who do not meet the criteria

(biscuits and cakes). The control products will range from 305-360 kcal (1286-1516 kJ), while the intervention products will range from 242-326 kcal (1013-1368 kJ) (full product nutritional information in **Supplemental Material 2**). Intervention and control products will be matched for sweetness intensity, flavour and physical appearance.

The 2 individual S&SEs selected based on published literature were Neotame and Stevia Rebaudioside M (in the biscuits and cakes) and 2 further S&SE blends were Sucralose/Acesulfame K blend and Mogroside V/Stevia Rebaudioside M blend (in yoghurt, chocoloate and cereal), selected based on the results of a preliminary study using a beverage matrix (manuscript in preparation).

Data Collection and Outcomes

Table 1 Details at which time point(s) data are collected at the CID.

Table 1: Data Collection and Timepoints for each CID

		Baseline or 0' (fasting)	10'	15'	20'	30'	45'	60'	120'	180'	Next day
Primary endpoint	Subjective appetite (VAS for hunger, desire to eat, fullness, prospective food consumption)	X	X		X	X	X	X	X	X	
Behavioural endpoints	Food preference & reward (LFPQ)	X			X						
	Food cravings (CoEQ)	X									
	Energy intake (24-hour dietary recall)										X
	Expected satiety	X (1 bite)									
	Sensory-specific satiety	X (1 bite)	X								
	Other appetite ratings (e.g., thirst, nausea, bloating. appetite for something sweet/savoury)	X	X		X		X	X	X	X	
Metabolic endpoints	Glucose and insulin	X	X	X		X		X	X		
	Pancreatic polypeptide (PP)*	X	X	X		X					
	GLP-1 and ghrelin	X				X		X			
	Lipaemia (triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol)	X				X		X	X		
Health endpoints	Liver function (ALT, AST, GGT, FL index, TyG index)	X							X		
	HbA1c	CID1 & 6									
	24-hour GI side effects (self-report)										X

* Timepoints for PP are earlier for yoghurt study

Abbreviations: ALT - alanine transaminase, AST – aspartate transaminase, GGT - gamma-glutamyltransferase, FL index - fatty liver index, TyG index - triglycerides and glucose index VAS - visual analogue scale, LFPQ - Leeds Food Preference Questionnaire, CoEQ - Control

of Eating questionnaire, GLP-1- Glucagon-like peptide 1, HDL - High density lipoprotein, LDL - Low density lipoprotein, HbA1c - Haemoglobin A1c, GI - Gastrointestinal, CID - Clinical Investigation Day.

Primary Outcome

This trial has one primary outcome which is the iAUC for the 180-minute composite appetite score based on hunger, fullness (reverse scored), desire to eat and prospective food consumption (36). These subjective appetite ratings will be measured throughout the CIDs using VAS on the QDP. The trapezoid method will be used for the calculation of iAUC (25).

Secondary Outcomes

Food preference and reward

Food preference and food reward will be measured at all CIDs using the LFPQ (32). Changes will be determined by comparing the relative preference/food choice, explicit liking and implicit wanting for high-fat sweet, low-fat sweet, high-fat savoury and low-fat savoury foods, and fat/sweet appeal bias scores in the fed and hungry states between the reformulated and control products.

Food cravings

Food cravings will be determined at all CIDs by craving control, craving for sweet and savoury scores from the CoEQ (29), which is a 21-item questionnaire with responses recorded on a 100-point VAS (1 item allows for text response).

Energy intake

Energy intake will be measured by a 24-hour dietary recall (using the multiple pass method (37)), which will be conducted by a trained dietitian or research staff over the telephone. Participants will be asked to recall all food and drink consumed during the 24-hour period since leaving the laboratory. Participants will receive training on reporting food portions using the Australian Health Survey Food Model Booklet (38) or similar culturally adapted resources.

Compensatory eating behaviour will be determined from the analysis of the 24-hour dietary interview data using energy intakes calculated with national nutritional software. The following variables will be considered: 1) Energy and macronutrient distribution and 2) Percent energy

compensation (%EC), defined as the adjustment of energy intake (EI) provoked by the intervention products (39), (see **Supplemental Material 3** for further information).

Expected satiety and sensory specific satiety

Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (33, 40) and sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire (34) after one bite and full consumption (10') of the product. Responses to both questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).

Other behavioural ratings

Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).

Biochemical measures

Blood for plasma analyses will be centrifuged at 1500 *g* at 4°C for 10 minutes immediately after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately after collection. Plasma and serum aliquots will be stored at -80°C until shipment for analyses to Bioaitriki Laboratories (central lab) in Athens, Greece.

Insulin concentrations will be determined by chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system. Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay (Abbott) which consists of two separate concentration measurements: glycated haemoglobin (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-cholesterol will be determined by an accelerator selective detergent method (Ultra HDL assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye formation method (Direct LDL assay, Abbott). AST and ALT will be determined by enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-

carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated according to information provided in **Supplemental Material 5**.

Gastrointestinal (GI) side effects

Any reported unusual GI side effects, including abdominal pain/cramps, heartburn, stomach acid/reflux, nausea, vomiting, abdominal rumbling, bloating, belching, excess gas/wind, bowel movements, stool type, etc. during the study will be recorded at the phone call the day after each CID and each day during the at-home intervention in a booklet including the Bristol Stool Form Scale (41). The GI symptoms check has been based on the validated Gastrointestinal Symptoms Rating Scale (GSRS) tool (42).

STATISTICAL ANALYSIS PLAN

Per protocol analysis will include participants that completed all 6 CIDs and had a level of adherence to the product consumption >80%. The main evaluations for this trial will be to investigate differences between the intervention products (2 no added/reduced sugar reformulated S&SE products and 1 sucrose-sweetened control). Where this is not appropriate for some of the secondary outcomes, descriptive analyses will be used to interpret differences. Data will be pooled across the split-site (Leeds and Lyon) study using the biscuit matrix. Data will be presented as means and standard deviation. Outcome variables will be checked for normality and transformed where necessary. To account for any missing data, analyses will be conducted using linear mixed models. Models will compare S&SE product conditions vs. sucrose control in a 3 (S&SE1, S&SE2, sucrose control) x 2 (exposure day 1 and exposure day 14) within-subject design. Model parameters will be adjusted to obtain the best model fit. Adjustments for covariates (e.g. age, gender, BMI, intervention site, compliance, protocol deviations, adverse events and concomitant medication) will be applied as necessary, e.g. in the event that they influence outcomes. Analyses will be reported as both unadjusted and adjusted models. The American Statistical Association's policy statement on p-values (43) advises that all p-values from specified statistical models be reported along with point estimates, effect size and confidence intervals to help interpret the compatibility of the data with the study outcomes, therefore this procedure will be followed. Otherwise, the level of significance will be set at 0.05.

Safety analysis

Information relating to adverse events (including events relating to GI side effects) and concomitant medication will be tabulated and summarised descriptively.

ETHICS AND MONITORING

Each intervention site will obtain ethical approval from their local ethical committee. All study procedures will be conducted in accordance with the Helsinki Declaration and the study protocol has been registered in a public database (clinicaltrials.gov NTC04633681; **Supplemental Material 6**). To the extent relevant and reasonable International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines will be used, and standard operating procedures (SOP) will be developed to facilitate the same performance and compliance with the protocol in each centre. All personal data is handled confidentially and stored in accordance with applicable law, GDPR and local laws (see **Supplemental Material 7**). All participants will receive written and oral information about the study and only trained study personnel will provide information, monitor and attest signing of the informed consent form. Where required, monitoring of intervention sites will be performed during the study by the University of Navarra depending on local regulations.

TRIAL STATUS

The COVID pandemic had a large impact on access to infrastructure and services across all intervention centres. For example, research was halted in some institutions or fewer participants could be scheduled per visit (restrictions related to distance and number of social contacts), recruitment of new staff was frozen, new risk assessments were required, ethical review processes were restricted or extremely prolonged because COVID-related protocols were prioritized, procurement of supplies, consumables and services was suspended, and IT and administrative support was restricted. Further, face-to-face clinical work was put under strain. There were also challenges regarding staff and volunteer sickness plus overall volunteer reluctancy to engage in clinical trials affecting the speed of recruitment and testing. Nevertheless, recruitment opened in May 2021 for the trial at the Leeds and Lyon intervention centres using the biscuit matrix, with last participant last visit completed in June 2022 for Leeds and expected by October 2022 for Lyon. Recruitment for the trial at Lyon using the cake matrix opened in February 2022. The trials at Liverpool and Pamplona started recruiting in Spring 2022, and Copenhagen are still awaiting ethical approval (August 2022).

PUBLICATION

After completion of the study, the findings will be submitted for publication in an international peer-reviewed scientific open access journal and other relevant media. Research data from the trial will be deposited in an open access online research data archive (for example Zenodo).

FUNDING STATEMENT

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COMPETING INTERESTS STATEMENT

JCGH, JAH and CAH and are in receipt of research funding from the American Beverage Association. CAH has received honoraria from the International Sweeteners Association. ARA has received honoraria from Unilever and the International Sweeteners Association. CH's research centre provides consultancy to and has received travel funds to present research results from organisations supported by food and drink companies. CS is a paid employee of Cargill, Inc.

AUTHORS' CONTRIBUTIONS

The SWEET EU-project was initiated by JH, JCGH and AR. The protocol for the present SWEET work package intervention trial was written by CG, CHa, EA-R, SN-C, CHo, J-AN, JAM, CS, EB, EF, HM, KB and GF, with all contributing to the design of the trial along with BO'H, DO'C, MW, MA, MN, CR. JAM, GF, J-AN, AR and CHa are principal investigators (PI) at the 5 intervention sites. CG and BO'H drafted the manuscript and EA-R, SN-C, MW, CS, LK, AR, KB and GF critically reviewed the manuscript. All authors read and approved the final manuscript. Responsible author is CG.

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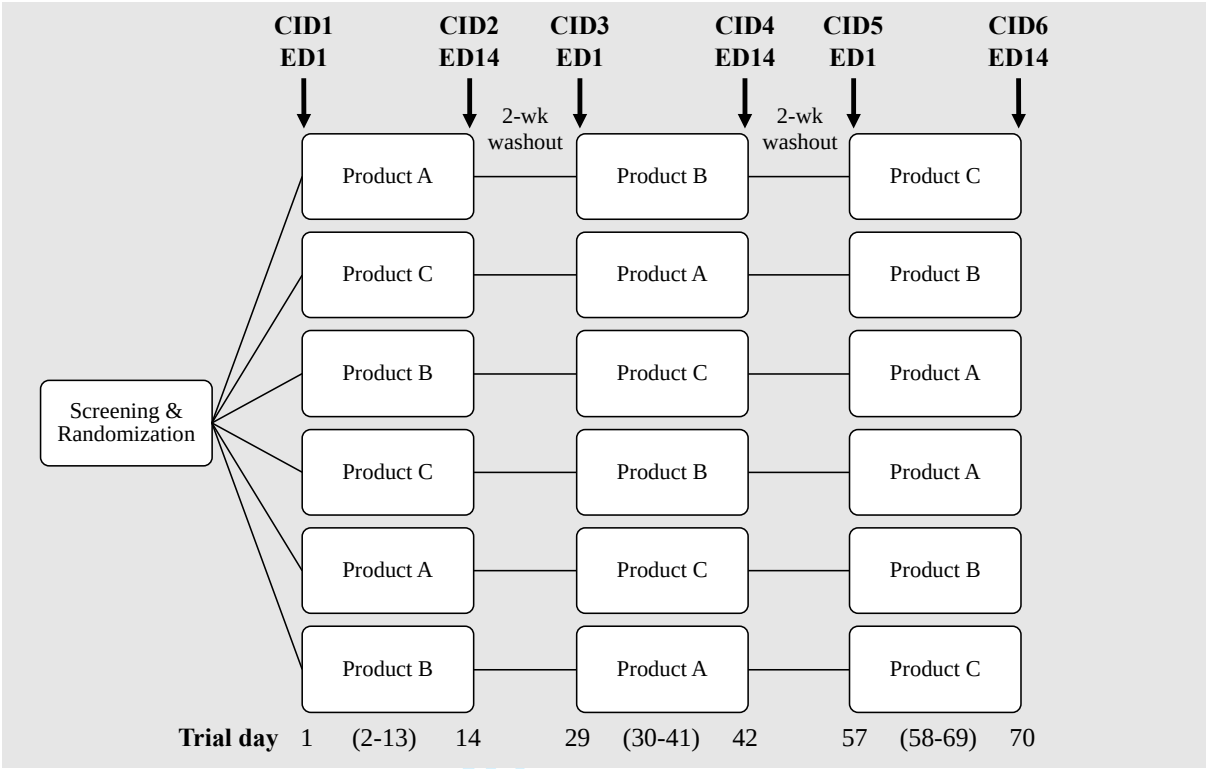

Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to order one will consume product A in the lab on clinical investigation day (CID) 1/exposure day (ED) 1 and then every day at home until CID2/ED14 when it is consumed in the lab again. After a 2-week washout, the participant returns to the lab and repeats the study block with product B, followed by another 2-week washout, followed by the final study block with product C.

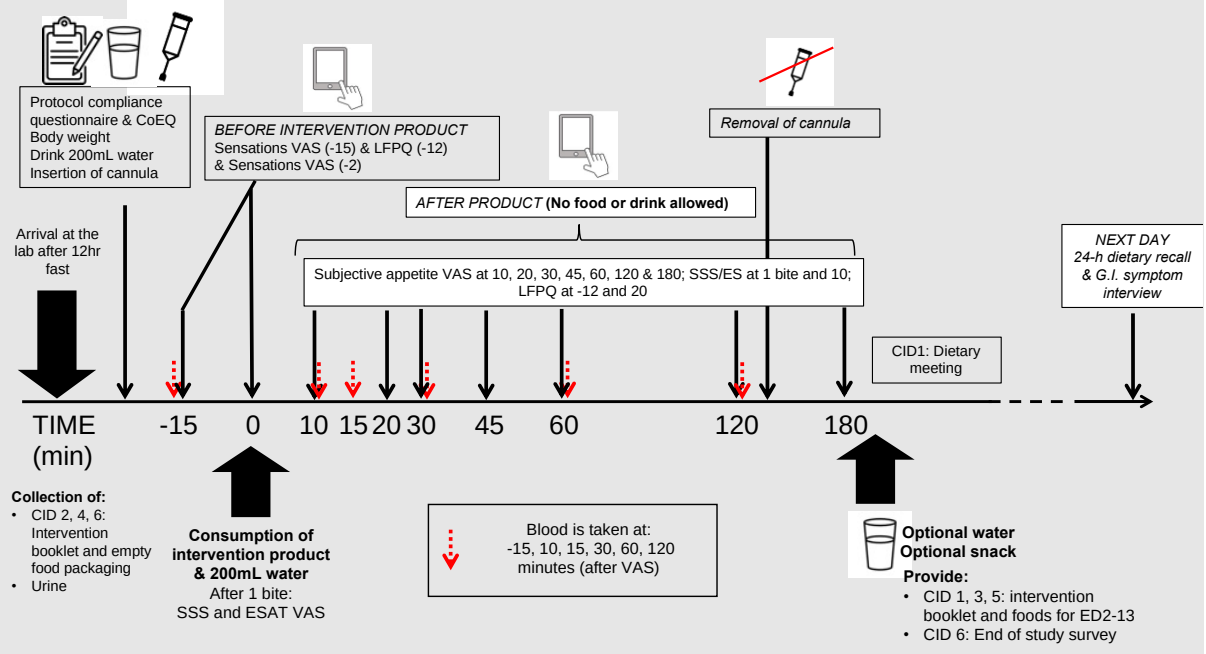

Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix. CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.

Supplemental Material 1: Exclusion Criteria

General Criteria

- Blood donation < 3 month prior to study or for full duration of the study.
- Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g., veganism) or history of anaphylactic reaction to any food.
- Likelihood for disordered eating defined as a score ≥20 on the EAT-26 test
- Currently dieting to lose weight.
- Having lost or gained >4.5 kg in the last 3 months.
- Smoking or having quit <3 months prior to study.
- Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months.
- Performing >10 h of intense physical activity per week in the last 3 months.
- Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift.
- Self-reported use of drugs of abuse within the previous 12 months.
- For women: Pregnancy, lactation.
- Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study).
- Insufficient communication in the national language.
- Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen.
- Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician.
- Simultaneous participation in other relevant clinical intervention studies.
- Previous university or college training related to eating behaviour research.

Medical conditions as known by the person

- Self-reported eating disorders.
- Diagnosed anaemia.
- Diagnosed diabetes mellitus.
- Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer.
- Active inflammatory bowel disease, celiac disease, chronic pancreatitis, or other disorder potentially causing malabsorption.
- History of G.I. surgery with permanent effect (i.e., surgical treatment of obesity).
- Medical history of CVD (e.g., current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease).
- Significant liver disease, e.g., cirrhosis (fatty liver disease allowed).
- Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed).
- Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3 months.
- Psychiatric illness (e.g., major depression, bipolar disorders).

Medication

- Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety, or body weight incl. food supplements.

Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months.

- Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months).

Supplemental Material 2: Product Ingredients List

Table 1: Proposed energy and nutrient composition of the intervention products

	Control		Reformulation	
Cake with fruit filling	Per 100g	Per portion (85g/ 3 cakes)	Per 100g	Per portion (85g/ 3 cakes)
Energy (kcal)	391	332	343	292
Energy (kJ)	1638	1392	1427	1213
Fats (g)	16.6	14.1	16.5	14.0
Sat. fats (g)	1.7	1.4	1.7	1.4
Carbs (g)	56.8	48.2	57.0	48.4
Sugars (g)	28.3	24.1	1.3	1.1
Polyols (g)	3.7	3.1	28.4	24.1
Fibre (g)	1.4	1.2	1.3	1.1
Proteins (g)	5.7	4.8	5.7	4.8
Salt (mg)	0.4	0.3	0.4	0.3
Biscuit	Per 100g	Per portion (3 biscuits)	Per 100g	Per portion (3 biscuits)
Energy (kcal)	423	360	384	326
Energy (kJ)	1783	1516	1609	1368
Fats (g)	11.2	9.5	11.5	9.8
Sat. fats (g)	7.11	6.0	7.33	6.2
Carbs (g)	75.9	64.5	76.2	64.8
Sugars (g)	24.7	21.0	1.8	1.5
Polyols (g)	3.7	3.1	22.7	19.3
Fibre (g)	0.7	0.6	2.4	2.0
Proteins (g)	6.5	5.5	6.6	5.6
Salt (mg)	0.7	0.6	0.7	0.6
Creamy yoghurt	Per 100g	Per portion (135g 1 serving)	Per 100g	Per portion (135g 1 serving)
Energy (kcal)	226	305	180	242
Energy (kJ)	943	1286	750	1013
Fats (g)	18.5	25.0	16.3	22.0
Sat. fats (g)	8.3	18.7	8.3	18.7
Carbs (g)	8.12	10.96	1.71	2.31
Sugars (g)	8.12	10.96	1.71	2.31
Polyols (g)	0.00	0.00	0.00	0.00
Fibre (g)	0.00	0.00	0.00	0.00
Proteins (g)	6.50	8.78	6.50	8.78
Salt (mg)	0.14	0.32	0.14	0.32
Chocolate	Per 100g	Per portion (60g 1 bar)	Per 100g	Per portion (60g 1 bar)
Energy (kcal)	500	325	477	310
Energy (kJ)	2098	1364	2004	1303
Fats (g)	31	20	31	20
Sat. fats (g)	18	12	18	12
Carbs (g)	46	30	46	30
Sugars (g)	45	29	31	20
Polyols (g)	0	0	12	8
Fibre (g)	8	5	8	5
Proteins (g)	5	3	5	3
Salt (mg)	5	3	8	5
Honey ball breakfast cereal	Per 100g	Per portion (60 g of cereals + 125 ml (121 g) of milk)	Per 100g	Per portion 60 g of cereals + 125 ml (121 g) of milk)
Energy (kcal)	173	320	153	283
Energy (kJ)	731.2	1353.8	641.7	1187.2
Fats (g)	3.26	6.03	3.16	5.84
Sat. fats (g)	1.64	3.04	1.63	3.01
Carbs (g)	31.17	57.66	25.59	47.33

Sugars (g)	12.03	22.26	5.02	9.28
Polyols (g)	0	0	4.86	9.00
Fibre (g)	0.86	1.60	6.64	12.28
Proteins (g)	4.38	8.11	4.21	7.79
Salt (mg)	0.35	0.64	0.33	0.60

Supplementary Material 3 – Energy Intake Calculation

Percent energy compensation (%EC) was derived from the dietary recall data as previously reported by Zandstra et al.[1], and Almiron-Roig et al[2]. Briefly, %EC was calculated as:

$$\%EC = [\ (EI_{\text{Control Product}} - EI_{\text{Reformulated Product}})/|EP|]*100$$

where EI represents the cumulative energy intake 24-h post consumption under the control product or under the reformulated product conditions, excluding the energy of the product itself. EP (as an absolute value) represents the difference in energy between the full-energy-containing preload (i.e., control product) and the lower-energy-containing preload (i.e., reformulated products). For example, if the control product has a value of 325 kcal and the reformulated product has a value of 250 kcal, then EP=325-250 or 75 kcal).

1. Zandstra, E. H., Mathey, M. F., Graaf, C., & van Staveren, W. A. (2000). Short-term regulation of food intake in children, young adults and the elderly. *European Journal of Clinical Nutrition, 54*(3), 239-246. http://www.ncbi.nlm.nih.gov/pubmed/10713747

2. Almiron-Roig, E., Palla, L., Guest, K., Ricchiutu, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews, 71*(7), 458-473. https//doi.org/10.1111.nure.12048

Supplemental Material 4: Questions used to assess product taste text, subjective appetite sensations, sensory specific satiety and expected satiety

Food Taste Test (FTT) (Conducted at screening)

Screen 1

You will now be presented with a food that we will ask you to evaluate. Please follow the instructions as they appear on the screen.

Screen 2

1. Take a mouthful of the food provided.
2. Chew while counting to 5.
3. Swallow.
4. Answer the question by moving the arrow to the left or to the right.

How **pleasant was this food**?

Not at all pleasant — Extremely pleasant

Screen 3

Thank you. This is the end of the taste test.

Please call the investigator after submitting your answer.

Subjective Appetite Questions (used during all CIDs)

Considering how you feel **right now**, give your answer to each of the following questions by moving the arrow to the left or to the right at the point that best represents your experience. The list below is the complete list of questions used via visual analogue scales.

1. How **hungry** do you feel?
2. How **full** do you feel?
3. How **thirsty** do you feel?
4. How strong is your **desire to eat**?
5. How **much** do you think you could **eat** right now?
6. How **nauseous** do you feel?
7. How **bloated** do you feel?
8. How strong is your appetite for something **savoury**?
9. How strong is your appetite for something **sweet?**

Sensory Specific Satiety Questionnaire (assessed after 1 bite and after consumption of product)

After 1 bite:
Please take a bite of the food and keep the food in your mouth while rating the food. Swallow the food only when your rating is complete.

How **pleasant** is the taste of the food right now?

At 10 minutes:
How **pleasant** is the taste of the food now that you have finished eating it?

Expected Satiety (ESAT) (assessed after 1 bite and after consumption of product)[1]

After 1 bite:
After having taken 1 bite of the food and looking at the whole food portion, how much will this portion of food stop you from feeling hungry between meals?

At 10 minutes after full consumption and after SSS rating:
How much will this food stop you from feeling hungry between meals?

Supplemental Material 5: Fatty liver index and triglyceride glucose index calculation

Calculation of Fatty Liver Index:

Some of the blood parameters will be used to calculate a Fatty Liver index (FL) using the formula of Bedogni et al [1],with measured values for BMI, fasting TG (mg/dL), fasting GGT (U/L) and waist circumference (cm), as follows:

$$FLI = (e^{0.953*\log e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e(\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) / (1 + e^{0.953*\log e(\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e(\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) * 100$$

Calculation of Triglyceride Glucose Index:

The formula of Simental-Mendía et al.[2] will be measured with measured fasting TG (mg/dL) and fasting glucose (mg/dL), by dividing the Ln of the TG *glucose product by 2:

TyG index = Ln [(fasting triglycerides) (mg/dL) x fasting glucose (mg/dL)] / 2

1. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterology*, *6*(1), 33. https://doi.org/10.1186/1471-230X-6-33

2. Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, *6*(4), 299–304. https://doi.org/10.1089/met.2008.0034

Protocol for acute and repeated dose impact of sweeteners and sweetness enhancers on appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-over, randomised, controlled trial in people with overweight/obesity. The SWEET project.

Supplemental Material 6: Trial Registration Data Set

Trial registration data	
Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT04633681
Date of registration in primary registry	November 2021
Secondary identifying numbers	N/A
Source(s) of monetary or material support	European Union Horizon 2020 Program
Primary sponsor	European Union Horizon 2020 Program
Secondary sponsor(s)	N/A
Contact for public queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Contact for scientific queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Public title	Impact of Sweeteners on Behaviour, Physiology & Health (SWEET-WP2-P2)
Scientific title	Acute and Repeated Impact of Sweeteners and Sweetness Enhancers on Food Behaviour, Physiology & Health (SWEET Work Package 2 Phase 2)
Countries of recruitment	Denmark, France, Spain, United Kingdom
Health condition(s) or problem(s) studied	Eating Behaviour
Intervention(s)	Consumption of food product with sweetener and sweetness enhancer Consumption of sucrose-sweetened control food product
Key inclusion and exclusion criteria	Ages eligible for study: ≥ 18 ≤60 years ; Sexes eligible for study :both; Accepts healthy volunteers: yes
	Inclusion criteria: BMI 25-35kgm2; Use of contraceptive methods or not planning to become pregnant for the duration of the study (women only); Regular consumption of sugar-containing foods and willing to consume sugar and artificially-sweetened food products; Liking of the intervention foods defined by a response of 'Yes' for the product during the pre-screening interview and a score of 40% or above on the Liking Visual Analogue Scale for the sucrose-sweetened control product; Able to participate on the Clinical Investigation Days during normal working hours; Healthy as determined from the self-reported medical history or when a clinical condition exists, when this is considered to be irrelevant (i.e. not influencing study outcomes) for the study by the study medical doctor; Consuming breakfast regularly (at least 5 days per week); Able to understand and be willing to sign the informed consent form, and to follow all the study procedures and requirements; Capacity to store at-home intervention quantity of intervention product
	Exclusion criteria: Blood donation < 3 month prior to study or for full duration of the study; Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g. veganism) or history of anaphylactic reaction to any food; Likelihood for disordered eating defined as a score ≥20 on the Eating Attitudes Test; Currently dieting to lose weight; Having lost or gained >4.5 kg in the last 3 months; Smoking or having quit <3 months prior to study; Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months; Performing >10 h of intense physical activity per week in the last 3 months; Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift; Self-reported use of drugs of abuse within the previous 12 months; Pregnancy, lactation (women only); Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study); Insufficient communication in the national language; Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen; Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician; Simultaneous participation in other relevant clinical intervention studies; Previous university or college training related to eating behaviour research; Self-reported eating disorders; Diagnosed anaemia; Diagnosed diabetes mellitus; Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer; Active inflammatory bowel disease, coeliac disease, chronic pancreatitis or other disorder potentially causing malabsorption; History of G.I. surgery with permanent effect (i.e. surgical treatment of obesity); Medical history of Cardiovascular Disease (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease); Significant liver disease, e.g. cirrhosis (fatty liver disease allowed); Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed); Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3months; Psychiatric illness (e.g. major depression, bipolar disorders); Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety or body weight incl. food supplements. Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for

	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering medication, if the dose has changed during the last3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months)
Study type	Interventional Allocation: Randomised. Double-blind, within-subjects, cross-over trial Primary purpose: Evaluation Phase: N/A
Date of first enrolment	April 2021
Target sample size	213
Recruitment status	Recruiting
Primary outcome(s)	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
Key secondary outcomes	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers: Glucagon-like peptide-1 (GLP-1)Incremental area under the curve for blood GLP-1 concentrations in response to each product (120 min post intake);Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product (120 min post intake).

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FASt
Food Acceptance Study

Institutional logo

Informed Consent Form

Title of research project: Food Acceptance Study (FAST) [or include title in national language]

I confirm that: (please initial next to each statement to show you agree)

I have obtained written and oral information about the research project and I am informed about the aim, methods, benefits and risks of participating in the study.	
I have read and have understood the information sheet [version number] for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that taking part in the study involves completing a screening visit plus 6 clinical investigation days during which I will need to consume foods, provide blood [urine, and faeces] samples and fill in questionnaires.	
I understand that I will not be able to donate blood for the duration of my participation in the study.	
I understand that my participation is voluntary and that I am free to stop taking part and can withdraw from the study prior to anonymization of the data (1st March 2023) without giving any reason and without my rights being affected.	
I understand that I can ask for access to the data I provide and I can request the destruction of that data at any time prior to anonymization of the data [add date]. I understand that after anonymization of the data, I will no longer be able to request access to or withdrawal of the data I provide.	

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I understand that the data, including any identifiable data I provide will be held securely and in line with data protection requirements at [add institution].	
I understand that pseudo-anonymised data (including my participant number) will be sent to other partners within the larger EU project for testing and analysis.	
I understand that pseudo-anonymised data may also be shared with other researchers who are not working on this study for future research purposes in the public interest.	
I understand that fully anonymised data (after destruction of the ID-log) will be made available to the public (open access). As a result other external organisations or researchers will be also able to access these anonymised data for future research purposes.	
I understand that my anonymised data will be retained indefinitely on password-protected computers at [add institution].	
I consent to participate in the above study.	
[Remove if not applicable] I consent that my biological material will be stored in a research biobank at the [enter University].	
I have received a copy of this informed consent form as well as a copy of the Participant Information Sheet ([version number]) and a copy of the General Data Protection Regulation information sheet ([version number]).	

Participant name: _____

Date: _____ Signature:_____

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In case new information that has substantial influence on your health emerges from the research project, you will be informed. If you would prefer **not** to be informed about such information please mark it here _____ (insert X).

Consent from the study staff that provided the oral information:

I declare, that the participant has received both written and oral information about the research project.

I declare to the best of my knowledge and belief that the participant has received sufficient information to decide to participate in the research project.

Study staff name: _____

Date: _____ Signature: _____

National project identification: [include e.g. ethical approval number from ethical committee and date of approval]

FASt

Food Acceptance Study

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General Data Protection Regulation (GDPR) information for study participants

What data will be collected, how will they be used and who will see them?

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In relation to your participation in the Food Acceptance STudy (FAST), a range of data will be collected from you. This document explains how your data will be used.

1. Which data will be collected and how?

The data collected includes information about health and personal data e.g. name, [civil registration number if relevant, include name/type], contact information, gender and ethnicity and biological material (i.e. blood[,urine and faeces]). The data is registered in a personal participant folder and/or in an electronic database.

Data and biological material that is sent from the intervention site to other laboratories or researchers will contain a participant number but never your name or any other personal identifying data.

The investigators and other data processors will ensure that the information collected about you is not accessed by unauthorized persons and that your identity is protected when the results of the study are published. The online questionnaire delivery platform collecting body sensations and other measures will only use basic cookies to enable the proper functioning of the program. No marketing or other tracking cookies will be used.

2. How will my data be stored?

The investigators and the data manager will take all necessary security precautions to ensure that any identifying information about you is kept confidential and stored securely in accordance with local law [include name/references] and EU Regulation.

You will be assigned a unique ID number which your data will be identified by throughout the study. All electronic forms of data will be protected with a password which can only be accessed by the researchers. All data recorded on paper will be locked in study-specific storage cabinets accessible only to the researchers on the project.

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3. How long will my data be stored for?

Pseudo-anonymised data (including your participant ID) will be stored for up to 5 years. At that point, the participant ID-log will be destroyed and the pseudo-anonymised data will become fully anonymised data.

Fully anonymised data (not including your participant ID) after completion of the study will be retained indefinitely in an open-access repository (see point 7. *Will my data be archived for use in other research projects in the future* below).

Your biological samples (i.e. blood[, urine and faeces]) will be stored temporarily at the intervention site in freezers at either -20 or -80°C and later sent to other specialist laboratories for analysis. Once the biological material has been analysed for results related to this study, it will be destroyed by the laboratory. Destruction will happen at the latest by [2025] (five years after the study has ended).

[A research biobank contains biological material that is stored for future related research. If you wish to donate any excess of your biological material from this study to the research biobank of the [add university or other identifying name]), you must state it separately below. The donation is completely voluntary and does not affect your participation in this study. Samples in the biobank of the [university name] consist of a small amount (e.g. 5 ml blood [amend as suitable]), and are stored at [include name of intervention site] in freezers at -80 ° C for a maximum of 15 years after the study has ended. In order to conduct new analyses of your biological material from this biobank in a new study, the national ethical committee must first approve the study. You can always contact the intervention site and ask to have your samples in the biobank destroyed, unless the samples have been totally anonymised beforehand, which means that no one, nor the principal investigator, can longer assign the material to you. Sample full anonymization will take place alongside full anonymisation of all other data, after 5 years from study termination at the latest.]

4. What measures are in place to protect the security and confidentiality of my data?

The site-principal investigator will store an identifier (ID)-log (“key”) that associates your participant number with your personal information. This ID-log

is stored at the intervention site separately from data and biological material in a locked room. Only a few relevant persons from the study staff have access to the ID-log including national and international authorities controlling clinical research projects e.g. the local ethical committee [and if relevant, include other local authorities]. The ID-log will be used to identify you in case it is relevant. The ID-log will be stored at the intervention site as long as it is relevant to have your contact information and for ethical and legal considerations related to the conduct of the study. The ID-log will be stored for a maximum of 5 years after the study has ended.

5. How will my data be used?

The data collected will be securely forwarded to a project data hub at the University of Navarra (Spain) and subsequently used for analysis.

In case you withdraw from the study, the data already collected from you may be used and included in the analyses if the researchers find it important for the quality of the study. Already collected data from you will therefore only be processed if it is fair and important for the study. However, you may request that your data are destroyed and no further use is made of them. Please note, it will not be possible to withdraw your data after the results have been processed (this may be approximately 3 months after the study has ended, or by the 1st of March 2023).

The results of the study, regardless of whether they are positive, negative or inconclusive, will be written-up and submitted for publication after the end of the study e.g. as a publication in a journal, a summary of the test results on the Internet or at www.clinicaltrials.gov. Published results do not contain any information that can identify you.

6. Who will have access to my data?

Your pseudo-anonymised data (including your participant number) will be securely sent to other partners within the larger EU project e.g. the University of Navarra (Spain) and the University of Surrey (UK) for analysis and your pseudo-anonymised biological material will be sent to partner laboratories, e.g. Bioiatriki S.A. (Athens, Greece) for testing and analysis. Data and biological material are

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only sent from the intervention site to other laboratories with your participant number and never your name or other personal identifying data.

Pseudo-anonymised data may also be shared with other researchers who are not working on this study for future research purposes in the public interest.

After full anonymisation (destruction of the participant ID-log) the data collected in this study will be made available to the public (open access) by depositing it in an open access repository or other related archive. As a result, other external organisations or researchers will be also able to apply to access these fully anonymised data.

7. Will my data be archived for use in other research projects in the future?

Yes. We will make the fully anonymised data available to other organisations or researchers by depositing it in an open access repository or other archive. It is important that you understand that your data will be completely anonymised for these purposes, therefore there will be no way that you can be identified.

8. How will my data be destroyed?

The ID-log for all data will be stored for a maximum of 5 years after the study has ended. After that point, the ID-log will be destroyed and all data will be fully anonymized. After full anonymisation data will be available to the public (open access).

Any excess biological material will be destroyed by the handling laboratories after the analyses have been completed [keep/remove: unless you have chosen to donate some to a biobank in your local country. If this is the case, biobank material will be destroyed after 15 years following the termination of the study (i.e. by 2035)].

☐**I confirm that I have read and agree to the above information about the handling, processing and storage of my personal information in this study**

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as well as data sharing procedures between external partners within this larger EU project and other external organisations.

[Please, consider if you want to donate excess material for the biobank:

☐ **Yes**, I want to donate potential excess biological material from me to a biobank.

☐ **No**, I do not want to donate potential excess biological material from me to a biobank.]

Participant´s signature:

_____

___

Date Name

Signature

Researcher´s signature:

_____

___

Date Name

Signature

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	12
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supplemental Material 6
Protocol version	#3	Date and version identifier	6
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	15
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7 and 15
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8-9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A: No safety/critical efficacy issues present

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15; Supplemental Material 4
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Data from participants who discontinue/ deviate from protocol not collected
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15; appendices
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A, no analysis included
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A, no analysis included

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A: No safety/critical efficacy issues present
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A: No safety/critical efficacy issues present
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A: No safety/critical efficacy issues present
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Appendices
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A: No safety/critical efficacy issues present
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
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Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendices

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12

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Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with overweight/obesity: The SWEET Project

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Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with overweight/obesity: The SWEET Project

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Abstract

Introduction: Intake of free sugars in European countries is high and attempts to reduce sugar intake have been mostly ineffective. Non-nutritive sweeteners and sweetness enhancers (S&SEs) can maintain sweet taste in the absence of energy, but little is known about the impact of acute and repeated consumption of S&SE in foods these products on appetite. This study aims to evaluate the effect of acute and repeated consumption of 2 individual S&SEs and 2 S&SE blends in semi-solid and solid foods on appetite and related behavioural, metabolic and health outcomes.

Methods and Analysis: A work package of the SWEET project, this study consists of 5 double-blind randomised cross-over trials which will be carried out at 5 sites across 4 European countries, aiming to have n=213. Five food matrices will be tested across 3 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends and no added sugar). Participants (body mass index (BMI) 25-35 kg/m^2; aged 18-60 years) will consume each formulation for 14 days. The primary endpoint is composite appetite score (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour postprandial incremental area under the curve during clinical investigation days on day 1 and 14.

Ethics and dissemination: The trial has been approved by national ethical committees and will be conducted in accordance with the Declaration of Helsinki. Results will be published in international peer-reviewed open access scientific journals. Research data from the trial will be deposited in an open access online research data archive.

Trial registration number: Clinicaltrials.gov: NCT04633681

Strengths and Limitations of this study:

- The trial is the first of its kind to investigate the effects of acute and repeated exposure to 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary (chocolate) and breakfast cereal.
- This trial includes a large range of outcomes across behaviour, physiology, and health from persons living in Northern, Central and Southern Europe.
- The COVID-19 pandemic resulted in changes to the design of the studies in the trial. Originally, all products were to be tested across 2 sites, but the reduced time frame means this is not possible for some products.

- Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will be reported descriptively in these 2 studies where appropriate.

BACKGROUND AND RATIONALE

The global increase in the prevalence of obesity and its associated diseases is driven by a range of internal factors, involving genetic, behavioural, and metabolic determinants along with permissive external factors from the physical, social, and public (nutritional) policy environment (1). One of the main behavioural drivers involves a diet too rich in energy intake relative to energy expenditure. Free sugar intake (derived from sugar added to foods and beverages by the manufacturer or consumer) is one nutritional component that has gained focus because of its low nutritional value (lack of vitamins, minerals or fibre) and its potential to add to overall energy consumed, facilitating weight gain (2), and potential altered appetite and endocrine responses to carbohydrates (sugars) relative to other macronutrients (3).

Simply restricting free sugars from the diet without substitution may reduce diet palatability or contribute to changes in sweet craving (4), particularly in women (5), resulting in poor acceptance.. The replacement of free sugars with non-nutritive sweeteners and sweetness enhancers (S&SEs) in food products is one method to reduce sugar intake while maintaining acceptance and palatability of the diet. S&SEs have increasingly been employed over recent years to reduce the energy and sugar content of foods; however, their impact on appetite- and health-related outcomes is somewhat unclear (6).

The effect of S&SEs on appetite is difficult to summarise due the types of studies, comparisons and S&SEs being used. One of the reasons for the current partial understanding of the appetitive and metabolic effects of S&SEs in humans is that different S&SEs are commonly assumed to have similar behavioural effects (6-8). Only recently, one 12-week investigation of 4 distinct S&SEs reported directionally dissimilar effects of saccharin compared to sucralose on body weight (9). A recent review comparing different S&SEs suggests that some have the potential to enhance appetite, but these effects do not follow through to subsequent energy intake (6). A recent meta-analysis detailed the impact of no- or low-energy sweetened preloads compared to conventionally sweetened preloads on *ad libitum* energy intake. They concluded that similar effects on energy intake were seen due to only partial compensation being evident (although total energy intake was lower in the no- or low-energy sweetener) (10). Furthermore, recent studies have highlighted that S&SEs may

reduce sweet food cravings and therefore reduce sugar intake (11) appetite and energy intake (12). Overall, there is currently insufficient evidence to make a clear conclusion about the effect of S&SEs on appetite and energy intake. Indeed, the 11 S&SEs that are currently approved for use in the EU are chemically heterogeneous and absorbed, metabolised, and excreted differently (13). Furthermore, most investigations of the relationship between S&SE intake and health outcomes have used beverages as the vehicle (14) ; these have recently been reviewed (15). Since the amount of S&SEs in the food supply is increasing in response to consumer demand (16) and policy (e.g., 'sugar taxes'(17, 18); EU initiatives (19, 20)), there is a pressing need to examine the appetite-related behavioural and metabolic consequences of consuming S&SEs particularly in semi-solid and solid food matrices. Furthermore, it should be acknowledged that differences between acute and longer-term effects of S&SEs may not be the same (21) and this needs investigating further.

AIMS AND OBJECTIVES

The main objective of this study is to evaluate the acute (1-day) and repeated (14-day) effects of 2 individual S&SEs and 2 S&SE blends in reformulated reduced or no added sugar food products (using 2 modulations of S&SEs per matrix) on appetite and related behavioural, metabolic and health outcomes in adult men and women with overweight or obesity.

The hypotheses are:

H_1 Consumption of no added/reduced sugar products reformulated with S&SEs will result in an altered incremental area under the curve (iAUC) appetite score, compared with the sucrose-sweetened control product after repeated compared with acute consumption.

H_2 There will be differences between the no added/reduced sugar and sucrose-sweetened formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-related (liver function and gastrointestinal (GI) side effects) outcomes.

TRIAL DESIGN

This study is part of the wider SWEET project (https://sweetproject.eu/) funded by the European Commission Horizon 2020 program. It is a multicentre double-blind, randomised

cross-over trial conducted across 5 intervention sites in 4 countries, with 3 product formulations (sucrose-sweetened control vs 2 individual S&SEs or S&SE blends) over 5 intervention product types (cake, biscuits, yoghurt, chocolate, and breakfast cereal matrices) aiming for a total of 213 completers. The protocol is reported as per the SPIRIT reporting guidelines (22). While this study addresses the short term impact of specific S&SEs vs added sucrose on appetite, another work package in the SWEET project will examine the long term (1-year) impact of a weight loss and weight maintenance intervention with or without S&SE as part of a healthy diet (23).

Sample Size Determination

The following calculations apply to the studies involving biscuit, yoghurt and chocolate matrices:

Primary outcome: Power calculations showed that to detect a minimum difference of 8 mm in appetite ratings on a 100 mm visual analogue scale (VAS) with 80% power, alpha 0.05, and based on a published within-subject SD of 14.4 mm in VAS measures (24), an overall sample of 53 completers (both sexes, same BMI group, across all centres) would be needed (25; p.30) per matrix. We expect this sample size will be sufficient to detect iAUC differences in the appetite response relative to control of around 8–10%, considered to be of practical relevance (26).

Secondary outcomes: Due to the number of secondary outcomes in this study, it was not feasible to conduct power calculations for all outcomes. However, we consulted published studies (e.g. Yeomans et al, 2016 (24)) which utilized a similar design and demonstrated effects of small nutritional manipulations on various gut peptides. In these studies, sample sizes ranged from 12-23 participants, giving us confidence that a sample of 53 participants per matrix should be sufficient to detect differences with clinical significance.

Due to the impact of the COVID-19 pandemic on the trial (detailed later), the cake and breakfast cereal studies were scaled down according to reduced feasibility at each intervention centre to n=24 (cake) and n=30 (breakfast cereal), and no blood samples will be collected in the cake study. The primary outcome will be reported descriptively in these 2 studies where appropriate and reflected in the study registration and protocol.

STUDY SETTING

This trial is conducted across 5 intervention sites in 4 countries across 3 regions of Europe, with each site testing a different product, whilst following the same protocol. Western Europe: Leeds (University of Leeds, UK) will test biscuits; Liverpool (University of Liverpool, UK) will test chocolate; Lyon (Centre de Recherche en Nutrition Humaine Rhône Alpes, France) will test biscuits and cakes. Northern Europe: Copenhagen (University of Copenhagen, Denmark) will test cereal. Southern Europe: Pamplona (University of Navarra, Spain) will test yoghurt. University's of Leeds and Navarra are the leaders of this work package, whilst University of Liverpool is the co-ordinating centre of the SWEET project in its entirety.

PATIENT AND PUBLIC INVOLVEMENT

During the study, research staff discuss with participants about their experiences of the clinical investigation days, examinations, participant information and written materials, etc. with the aim to understand and improve participants' experiences in current and future studies of this nature. This is also captured in an end of study survey.

ELIGIBILITY CRITERIA

Male and female adults aged 18-60 years, with a BMI 25-35 kg/m^2 are eligible. Participants are required to regularly consume sugar-containing foods and willing to consume sugar and sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-26) (27) score <20 and a short sweet food frequency questionnaire score ≥3 of 11, in addition to rating the control product as ≥40% on a 100-point liking VAS during the taste test and be willing to consume the product during the duration of the trial. All exclusion criteria are listed in **Supplemental Material 1**.

INTERVENTION

Each trial will begin with an initial exposure to one of the 3 assigned product formulations under controlled laboratory conditions (clinical investigation day, CIDs 1, 3, 5 – exposure day 1), followed by repeated daily consumption of the same product at home for 12 (± 2 days) and a final exposure in the laboratory on day 14 (± 2 days) under identical conditions as the first exposure (CIDs 2, 4, 6 – exposure day 14), resulting in all participants completing the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be followed by a wash out period of 14-21 days between formulations. During the at-home periods, participants will consume a portion of the product at a time and place they choose

using a substitution strategy for similar energy/sweetness foods in their habitual diet. Foods habitually consumed of an equivalent energy density/sweetness are identified using participant's answers to a food frequency questionnaire and an energy equivalent guide, with a decision making tree developed to identify the most suitable foods to substitute for each intervention product. This strategy is supported by advice and agreement from the research officer/dietitian. Compliance will be monitored by an intervention booklet completed daily and by return of empty food packaging. All food products are provided in the same blinded container/wrapping. The study duration for each participant will be a minimum of 70 days (plus 7-14 days allowance for extended washout to aid scheduling of CIDs).

Recruitment and Screening

Participants will be recruited via a variety of routes e.g., study databases, webpages, social media, posters, and flyers. Potential participants will be pre-screened using an online or telephonic pre-screening questionnaire in accordance with the inclusion and exclusion criteria. Candidates passing pre-screening will be invited to attend an information session, either online or in-person, where they will be given detailed information about the study and invited to participate in a Q&A session. Candidates who wish to participate in the study will provide written informed consent and sign a general data protection regulation (GDPR) form before being fully screened. The screening session will be performed in-person or online, and will consist of anthropometric measurements (height, weight, waist and hip circumference; all confirmed in-person at CID1 for participants being screened online); eligibility questionnaires (EAT-26 (27) and short sweet food frequency questionnaire); baseline questionnaires (A socio-demographic questionnaire, a questionnaire to assess habitual sweet food consumption, including regular and S&SE sweet foods (SWITCH sweet food frequency questionnaire (SWFFQ)) (28), a questionnaire to assess habitual physical activity (International Physical Activity Questionnaire (IPAQ)) (29) and a consumer perspective questionnaire); an eligibility taste test of the control intervention product here participants rated the pleasantness of the product on a 100mm VAS after taking one bite and chewing for 5 seconds (a score of >40mm was required for inclusion into the study). Candidates who pass the screening session will be enrolled into the study.

Randomisation and blinding

A Latin square design (6 treatment orders) will be used to randomly allocate product sequence into blocks of 6, as shown in Figure 1. The person responsible for generating the

sequences for all sites will not have any study related tasks e.g., inclusion or examination of participants. Each sequence will be stratified by sex (female/male) and age group (18-45 years/46-60 years). When feasible, a female/male ratio of minimum 60/40 was also considered to reflect the target population characteristics.

Blinding of the intervention products (reformulated and control products) will be done by the manufacturers. As such, blinding of the research and central laboratory staff will take place allowing for a double-blind intervention. Moreover, the statistical analyses of the main outcome variable will be done without breaking the intervention product-assignment code before the analyses are finalised.

[Figure 1]

Clinical investigation days

Prior to each CID, participants will be asked to consume a similar evening meal at the same time, before fasting for a minimum of 12 hours and a maximum of 15 hours. High intensity physical activity, alcohol, and coffee will not be allowed for 12 hours before arriving to the laboratory. Two glasses, approximately 500 mL, of non-carbonated water will be allowed during the fasting period. Participants will provide a spot urine sample collected max 24 hours before each CID and will be analysed for the presence of specific S&SEs.

The CID procedures are outlined in Figure 2. CID start times will be scheduled in the morning between 8.00 am and 10.30 am and participants will start all 6 CIDs at the same time. Participants will complete a protocol compliance questionnaire to verify the above requirements regarding diet, physical activity, etc. If compliance has been breached, staff will reschedule the CID (within the maximum 14 days allowed, otherwise a protocol deviation will be recorded). If compliance has been achieved, participants will then fill in the Control of Eating Questionnaire (CoEQ)(30) to assess cravings over the last 7 days, followed by a body weight measurement. Participants will consume 200 mL of water before having an intravenous cannula inserted into an antecubital vein by qualified personnel. A baseline fasting blood sample will be taken 15 minutes after insertion of the cannula. Once the fasting sample has been taken, participants will complete fasting subjective appetite ratings for hunger, fullness, thirst, desire to eat, prospective intake, nausea, bloating, appetite for

something savoury and for something sweet on a validated 100-point VAS accessed via a PC or a tablet (31, 32). These measures will be completed on an electronic Questionnaire Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be measured using a culturally adapted version of the Leeds Food Preference Questionnaire (LFPQ)(33) on a computer desktop. Appetite sensations measured by VAS will be repeated after the LFPQ and before the researcher brings the blinded intervention product served with 200 mL of water. The participant will be instructed to take one bite, then answer questions regarding sensory-specific satiety and expected satiety by VAS (34, 35). The participant will be asked to consume the rest of the product over a period of 5-10 minutes, depending on the time required to consume the matrix and asked to complete a set of appetite sensation questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture peak PP response (36) (yoghurt will be consumed faster than other products therefore blood samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30, 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In between measurements, participants will remain seated in a quiet area, free from food-related sensory stimuli and read/listen to music/use a computer (provided there is no material with reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete the participant will be offered water or a snack before leaving the laboratory. Participants will be reminded about the consumption of the products at home and that they will receive a phone call the next day to complete a 24-hour diet recall and report any GI symptoms. Following the end of the trial, participants will be debriefed if requested and offered the chance to complete a survey about the conduct of the study.

[Figure 2]

Intervention products

There will be 1 control product (sucrose-containing manufactured products) and 2 no added/reduced sugar reformulated products based on the same food matrix - including 2 modulations of S&SE content (inclusion as individual S&SE or S&SE blends). The reformulated products have a target of ≥30% reduction in energy and/or sugar to achieve the status of 'reduced sugar' by EU regulation No 1047/2012. This will not be possible in all products, therefore 'no added sugar' will be applied to products who do not meet the criteria

(biscuits and cakes). The control products will range from 305-360 kcal (1286-1516 kJ), while the intervention products will range from 242-326 kcal (1013-1368 kJ) (full product nutritional information in **Supplemental Material 2**). Intervention and control products will be matched for sweetness intensity, flavour and physical appearance.

The 2 individual S&SEs selected based on published literature were Neotame and Stevia Rebaudioside M (in the biscuits and cakes) and 2 further S&SE blends were Sucralose/Acesulfame K blend and Mogroside V/Stevia Rebaudioside M blend (in yoghurt, chocoloate and cereal), selected based on the results of a preliminary study using a beverage matrix (manuscript in preparation).

Data Collection and Outcomes

Table 1 Details at which time point(s) data are collected at the CID.

Table 1: Data Collection and Timepoints for each CID

		Baseline or 0' (fasting)	10'	15'	20'	30'	45'	60'	120'	180'	Next day
Primary endpoint	Subjective appetite (VAS for hunger, desire to eat, fullness, prospective food consumption)	X	X		X	X	X	X	X	X	
Behavioural endpoints	Food preference & reward (LFPQ)	X			X						
	Food cravings (CoEQ)	X									
	Energy intake (24-hour dietary recall)										X
	Expected satiety	X (1 bite)									
	Sensory-specific satiety	X (1 bite)	X								
	Other appetite ratings (e.g., thirst, nausea, bloating. appetite for something sweet/savoury)	X	X		X		X	X	X	X	
Metabolic endpoints	Glucose and insulin	X	X	X		X		X	X		
	Pancreatic polypeptide (PP)*	X	X	X		X					
	GLP-1 and ghrelin	X				X		X			
	Lipaemia (triglycerides, total cholesterol, HDL-cholesterol, and LDL-cholesterol)	X				X		X	X		
Health endpoints	Liver function (ALT, AST, GGT, FL index, TyG index)	X							X		
	HbA1c	CID1 & 6									
	24-hour GI side effects (self-report)										X

* Timepoints for PP are earlier for yoghurt study

Abbreviations: ALT - alanine transaminase, AST – aspartate transaminase, GGT - gamma-glutamyltransferase, FL index - fatty liver index, TyG index - triglycerides and glucose index VAS - visual analogue scale, LFPQ - Leeds Food Preference Questionnaire, CoEQ - Control

of Eating questionnaire, GLP-1- Glucagon-like peptide 1, HDL - High density lipoprotein, LDL - Low density lipoprotein, HbA1c - Haemoglobin A1c, GI - Gastrointestinal, CID - Clinical Investigation Day.

Primary Outcome

This trial has one primary outcome which is the iAUC for the 180-minute composite appetite score based on hunger, fullness (reverse scored), desire to eat and prospective food consumption (37). These subjective appetite ratings will be measured throughout the CIDs using VAS on the QDP. The trapezoid method will be used for the calculation of iAUC (26).

Secondary Outcomes

Food preference and reward

Food preference and food reward will be measured at all CIDs using the LFPQ (33). Changes will be determined by comparing the relative preference/food choice, explicit liking and implicit wanting for high-fat sweet, low-fat sweet, high-fat savoury and low-fat savoury foods, and fat/sweet appeal bias scores in the fed and hungry states between the reformulated and control products.

Food cravings

Food cravings will be determined at all CIDs by craving control, craving for sweet and savoury scores from the CoEQ (30), which is a 21-item questionnaire with responses recorded on a 100-point VAS (1 item allows for text response).

Energy intake

Energy intake will be measured by a 24-hour dietary recall (using the multiple pass method (38)), which will be conducted by a trained dietitian or research staff over the telephone. Participants will be asked to recall all food and drink consumed during the 24-hour period since leaving the laboratory. Participants will receive training on reporting food portions using the Australian Health Survey Food Model Booklet (39) or similar culturally adapted resources.

Compensatory eating behaviour will be determined from the analysis of the 24-hour dietary interview data using energy intakes calculated with national nutritional software. The following variables will be considered: 1) Energy and macronutrient distribution and 2) Percent energy

compensation (%EC), defined as the adjustment of energy intake (EI) provoked by the intervention products (40), (see **Supplemental Material 3** for further information).

Expected satiety and sensory specific satiety

Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (34, 41) and sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire (35) after one bite and full consumption (10') of the product. Responses to both questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).

Other behavioural ratings

Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).

Biochemical measures

Blood for plasma analyses will be centrifuged at 1500 *g* at 4°C for 10 minutes immediately after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately after collection. Plasma and serum aliquots will be stored at -80°C until shipment for analyses to Bioaitriki Laboratories (central lab) in Athens, Greece.

Insulin concentrations will be determined by chemiluminescent microparticle immunoassay (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system. Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay (Abbott) which consists of two separate concentration measurements: glycated haemoglobin (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-cholesterol will be determined by an accelerator selective detergent method (Ultra HDL assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye formation method (Direct LDL assay, Abbott). AST and ALT will be determined by enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-

carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated according to information provided in **Supplemental Material 5**.

Gastrointestinal (GI) side effects

Any reported unusual GI side effects, including abdominal pain/cramps, heartburn, stomach acid/reflux, nausea, vomiting, abdominal rumbling, bloating, belching, excess gas/wind, bowel movements, stool type, etc. during the study will be recorded at the phone call the day after each CID and each day during the at-home intervention in a booklet including the Bristol Stool Form Scale (42). The GI symptoms check has been based on the validated Gastrointestinal Symptoms Rating Scale (GSRS) tool (43).

STATISTICAL ANALYSIS PLAN

Per protocol analysis will include participants that completed all 6 CIDs and had a level of adherence to the product consumption >80%. The main evaluations for this trial will be to investigate differences between the intervention products (2 no added/reduced sugar reformulated S&SE products and 1 sucrose-sweetened control). Where this is not appropriate for some of the secondary outcomes, descriptive analyses will be used to interpret differences. Data will be pooled across the split-site (Leeds and Lyon) study using the biscuit matrix. Data will be presented as means and standard deviation. Outcome variables will be checked for normality and transformed where necessary. To account for any missing data, analyses will be conducted using linear mixed models. Models will compare S&SE product conditions vs. sucrose control in a 3 (S&SE1, S&SE2, sucrose control) x 2 (exposure day 1 and exposure day 14) within-subject design. Model parameters will be adjusted to obtain the best model fit. Adjustments for covariates (e.g. age, gender, BMI, intervention site, compliance, protocol deviations, adverse events and concomitant medication) will be applied as necessary, e.g. in the event that they influence outcomes. Analyses will be reported as both unadjusted and adjusted models. The American Statistical Association's policy statement on p-values (44) advises that all p-values from specified statistical models be reported along with point estimates, effect size and confidence intervals to help interpret the compatibility of the data with the study outcomes, therefore this procedure will be followed. Otherwise, the level of significance will be set at 0.05.

Safety analysis

Information relating to adverse events (including events relating to GI side effects) and concomitant medication will be tabulated and summarised descriptively.

ETHICS AND MONITORING

Each intervention site has obtained ethical approval from their local ethical committee. The following details the specific ethical committees and the reference numbers. University of Leeds School of Psychology (PSC-127, approved 19th November 2020), Comité de Protection des Personnes Nord-Ouest III (2021-42, approved 28th March 2022), Comité de Ética de la Investigación de la Universidad de Navarra (2021.205, approved 7th March 2022), The Ethical Committee, Region H. Denmark (application number H-21078447, submitted and have made comments back to review committee, final decision is imminent) and University of Liverpool Central University Research Ethics Committee D (10659, approved 14th April 2022). All study procedures will be conducted in accordance with the Helsinki Declaration and the study protocol has been registered in a public database (clinicaltrials.gov NTC04633681; **Supplemental Material 6**). To the extent relevant and reasonable International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines will be used, and standard operating procedures (SOP) will be developed to facilitate the same performance and compliance with the protocol in each centre. All personal data is handled confidentially and stored in accordance with applicable law, GDPR and local laws (see **Supplemental Material 7**). All participants will receive written and oral information about the study and only trained study personnel will provide information, monitor and attest signing of the informed consent form. Where required, monitoring of intervention sites will be performed during the study by the University of Navarra depending on local regulations.

TRIAL STATUS

The COVID pandemic had a large impact on access to infrastructure and services across all intervention centres. For example, research was halted in some institutions or fewer participants could be scheduled per visit (restrictions related to distance and number of social contacts), recruitment of new staff was frozen, new risk assessments were required, ethical review processes were restricted or extremely prolonged because COVID-related protocols were prioritized, procurement of supplies, consumables and services was suspended, and IT and administrative support was restricted. Further, face-to-face clinical work was put under

strain. There were also challenges regarding staff and volunteer sickness plus overall volunteer reluctancy to engage in clinical trials affecting the speed of recruitment and testing. Nevertheless, recruitment opened in May 2021 for the trial at the Leeds and Lyon intervention centres using the biscuit matrix, with last participant last visit completed in June 2022 for Leeds and expected by October 2022 for Lyon. Recruitment for the trial at Lyon using the cake matrix opened in February 2022. The trials at Liverpool and Pamplona started recruiting in Spring 2022, and Copenhagen are still awaiting ethical approval (August 2022).

PUBLICATION

After completion of the study, the findings will be submitted for publication in an international peer-reviewed scientific open access journal and other relevant media. Research data from the trial will be deposited in an open access online research data archive (for example Zenodo).

FUNDING STATEMENT

The present study is funded by the Horizon 2020 program: Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability (acronym: SWEET, grant no: 774293). The current study was initiated by Prof. G. Finlayson as part of the Work Package 2 of the SWEET project. The study receives funding from the Horizon2020 program (9 million Euros) to cover salary for project personnel, supplies, remuneration and dissemination of results. The amount is deposited in a project account subject to public revision. The funder has no role in the study design, interpretation of data or publication of material.

COMPETING INTERESTS STATEMENT

JCGH, JAH and CAH and are in receipt of research funding from the American Beverage Association. CAH has received honoraria from the International Sweeteners Association. ARA has received honoraria from Unilever and the International Sweeteners Association. CH's research centre provides consultancy to and has received travel funds to present research results from organisations supported by food and drink companies. CS is a paid employee of Cargill, Inc.

AUTHORS' CONTRIBUTIONS

The SWEET EU-project was initiated by JH, JCGH and AR. The protocol for the present SWEET work package intervention trial was written by CG, CHa, EA-R, SN-C, CHo, J-AN, JAM, CS, EB, EF, HM, KB and GF, with all contributing to the design of the trial along with BO'H, DO'C, MW, MA, MN, CR. JAM, GF, J-AN, AR and CHa are principal investigators (PI) at the 5 intervention sites. CG and BO'H drafted the manuscript and EA-R, SN-C, MW, CS, LK, AR, KB and GF critically reviewed the manuscript. All authors read and approved the final manuscript. Responsible author is CG.

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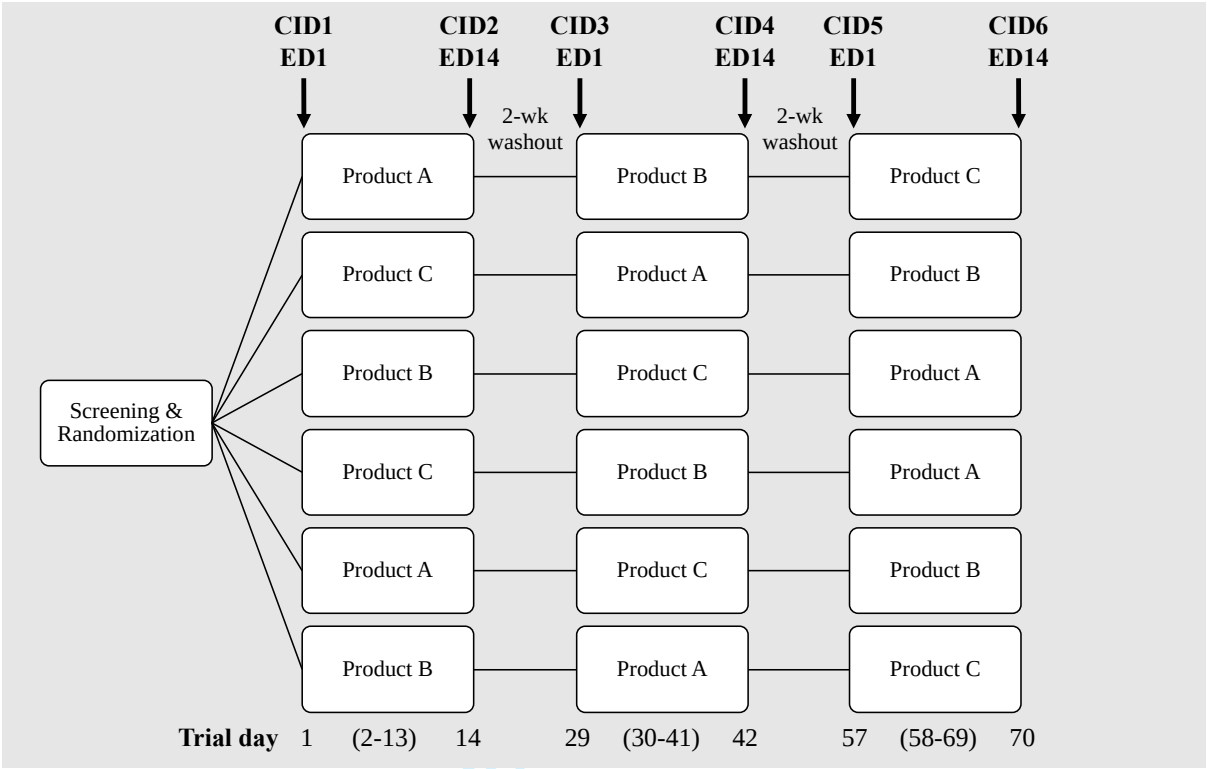
FIGURE LEGENDS

Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to order one will consume product A in the lab on clinical investigation day (CID) 1/exposure day (ED) 1 and then every day at home until CID2/ED14 when it is consumed in the lab again. After a 2-week washout, the participant returns to the

lab and repeats the study block with product B, followed by another 2-week washout, followed by the final study block with product C.

Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix. CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.



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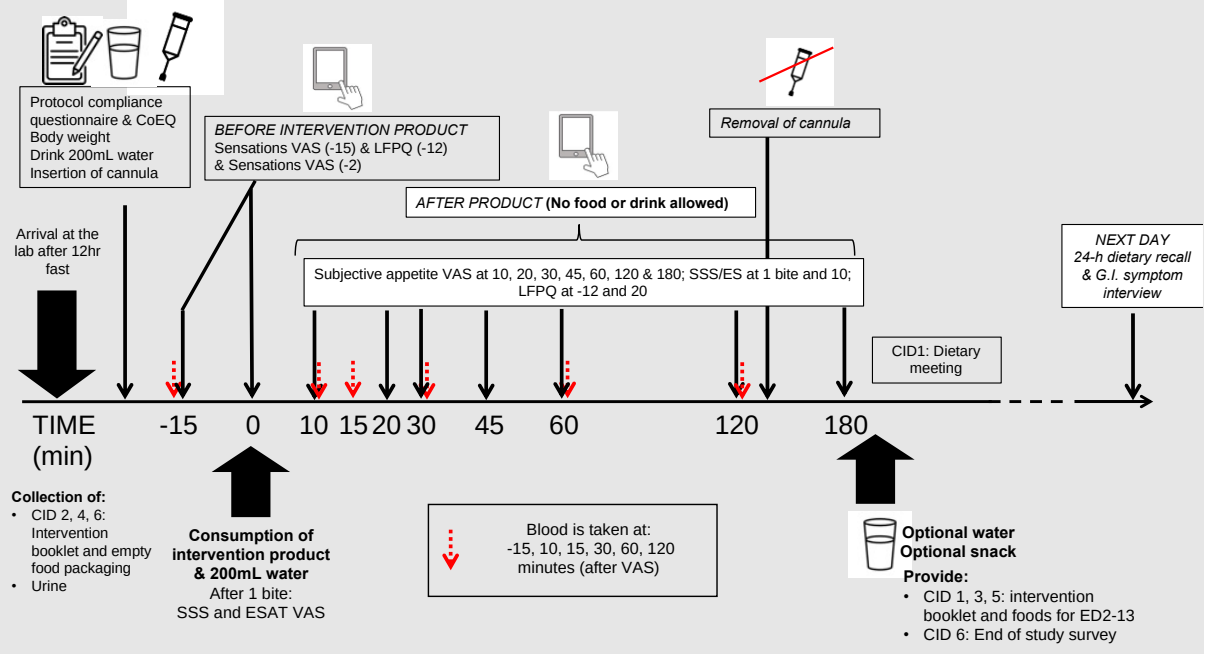

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Supplemental Material 1: Exclusion Criteria

General Criteria

- Blood donation < 3 month prior to study or for full duration of the study.
- Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g., veganism) or history of anaphylactic reaction to any food.
- Likelihood for disordered eating defined as a score ≥20 on the EAT-26 test
- Currently dieting to lose weight.
- Having lost or gained >4.5 kg in the last 3 months.
- Smoking or having quit <3 months prior to study.
- Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months.
- Performing >10 h of intense physical activity per week in the last 3 months.
- Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift.
- Self-reported use of drugs of abuse within the previous 12 months.
- For women: Pregnancy, lactation.
- Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study).
- Insufficient communication in the national language.
- Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen.
- Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician.
- Simultaneous participation in other relevant clinical intervention studies.
- Previous university or college training related to eating behaviour research.

Medical conditions as known by the person

- Self-reported eating disorders.
- Diagnosed anaemia.
- Diagnosed diabetes mellitus.
- Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer.
- Active inflammatory bowel disease, celiac disease, chronic pancreatitis, or other disorder potentially causing malabsorption.
- History of G.I. surgery with permanent effect (i.e., surgical treatment of obesity).
- Medical history of CVD (e.g., current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease).
- Significant liver disease, e.g., cirrhosis (fatty liver disease allowed).
- Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed).
- Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3 months.
- Psychiatric illness (e.g., major depression, bipolar disorders).

Medication

- Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety, or body weight incl. food supplements.

Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months.

- Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months).

Supplemental Material 2: Product Nutritional Information

Table 1: Proposed energy and nutrient composition of the intervention products

	Control		Reformulation	
Cake with fruit filling	Per 100g	Per portion (85g/ 3 cakes)	Per 100g	Per portion (85g/ 3 cakes)
Energy (kcal)	391	332	343	292
Energy (kJ)	1638	1392	1427	1213
Fats (g)	16.6	14.1	16.5	14.0
Sat. fats (g)	1.7	1.4	1.7	1.4
Carbs (g)	56.8	48.2	57.0	48.4
Sugars (g)	28.3	24.1	1.3	1.1
Polyols (g)	3.7	3.1	28.4	24.1
Fibre (g)	1.4	1.2	1.3	1.1
Proteins (g)	5.7	4.8	5.7	4.8
Salt (mg)	0.4	0.3	0.4	0.3
Biscuit	Per 100g	Per portion (3 biscuits)	Per 100g	Per portion (3 biscuits)
Energy (kcal)	423	360	384	326
Energy (kJ)	1783	1516	1609	1368
Fats (g)	11.2	9.5	11.5	9.8
Sat. fats (g)	7.11	6.0	7.33	6.2
Carbs (g)	75.9	64.5	76.2	64.8
Sugars (g)	24.7	21.0	1.8	1.5
Polyols (g)	3.7	3.1	22.7	19.3
Fibre (g)	0.7	0.6	2.4	2.0
Proteins (g)	6.5	5.5	6.6	5.6
Salt (mg)	0.7	0.6	0.7	0.6
Creamy yoghurt	Per 100g	Per portion (135g 1 serving)	Per 100g	Per portion (135g 1 serving)
Energy (kcal)	226	305	180	242
Energy (kJ)	943	1286	750	1013
Fats (g)	18.5	25.0	16.3	22.0
Sat. fats (g)	8.3	18.7	8.3	18.7
Carbs (g)	8.12	10.96	1.71	2.31
Sugars (g)	8.12	10.96	1.71	2.31
Polyols (g)	0.00	0.00	0.00	0.00
Fibre (g)	0.00	0.00	0.00	0.00
Proteins (g)	6.50	8.78	6.50	8.78
Salt (mg)	0.14	0.32	0.14	0.32
Chocolate	Per 100g	Per portion (60g 1 bar)	Per 100g	Per portion (60g 1 bar)
Energy (kcal)	500	325	477	310
Energy (kJ)	2098	1364	2004	1303
Fats (g)	31	20	31	20
Sat. fats (g)	18	12	18	12
Carbs (g)	46	30	46	30
Sugars (g)	45	29	31	20
Polyols (g)	0	0	12	8
Fibre (g)	8	5	8	5
Proteins (g)	5	3	5	3
Salt (mg)	5	3	8	5
Honey ball breakfast cereal	Per 100g	Per portion (60 g of cereals + 125 ml (121 g) of milk)	Per 100g	Per portion 60 g of cereals + 125 ml (121 g) of milk)
Energy (kcal)	173	320	153	283
Energy (kJ)	731.2	1353.8	641.7	1187.2
Fats (g)	3.26	6.03	3.16	5.84
Sat. fats (g)	1.64	3.04	1.63	3.01
Carbs (g)	31.17	57.66	25.59	47.33

Sugars (g)	12.03	22.26	5.02	9.28
Polyols (g)	0	0	4.86	9.00
Fibre (g)	0.86	1.60	6.64	12.28
Proteins (g)	4.38	8.11	4.21	7.79
Salt (mg)	0.35	0.64	0.33	0.60

Supplementary Material 3 – Energy Intake Calculation

Percent energy compensation (%EC) was derived from the dietary recall data as previously reported by Zandstra et al.[1], and Almiron-Roig et al[2]. Briefly, %EC was calculated as:

$$\%EC = [\ (EI_{\text{Control Product}} - EI_{\text{Reformulated Product}})/|EP|]*100$$

where EI represents the cumulative energy intake 24-h post consumption under the control product or under the reformulated product conditions, excluding the energy of the product itself. EP (as an absolute value) represents the difference in energy between the full-energy-containing preload (i.e., control product) and the lower-energy-containing preload (i.e., reformulated products). For example, if the control product has a value of 325 kcal and the reformulated product has a value of 250 kcal, then EP=325-250 or 75 kcal).

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Supplemental Material 4: Questions used to assess product taste text, subjective appetite sensations, sensory specific satiety and expected satiety

Food Taste Test (FTT) (Conducted at screening)

Screen 1

You will now be presented with a food that we will ask you to evaluate. Please follow the instructions as they appear on the screen.

Screen 2

1. Take a mouthful of the food provided.
2. Chew while counting to 5.
3. Swallow.
4. Answer the question by moving the arrow to the left or to the right.

How **pleasant was this food**?

Not at all pleasant — Extremely pleasant

Screen 3

Thank you. This is the end of the taste test.

Please call the investigator after submitting your answer.

Subjective Appetite Questions (used during all CIDs)

Considering how you feel **right now**, give your answer to each of the following questions by moving the arrow to the left or to the right at the point that best represents your experience. The list below is the complete list of questions used via visual analogue scales.

1. How **hungry** do you feel?
2. How **full** do you feel?
3. How **thirsty** do you feel?
4. How strong is your **desire to eat**?
5. How **much** do you think you could **eat** right now?
6. How **nauseous** do you feel?
7. How **bloated** do you feel?
8. How strong is your appetite for something **savoury**?
9. How strong is your appetite for something **sweet?**

Sensory Specific Satiety Questionnaire (assessed after 1 bite and after consumption of product)

After 1 bite:
Please take a bite of the food and keep the food in your mouth while rating the food. Swallow the food only when your rating is complete.

How **pleasant** is the taste of the food right now?

At 10 minutes:
How **pleasant** is the taste of the food now that you have finished eating it?

Expected Satiety (ESAT) (assessed after 1 bite and after consumption of product)[1]

After 1 bite:
After having taken 1 bite of the food and looking at the whole food portion, how much will this portion of food stop you from feeling hungry between meals?

At 10 minutes after full consumption and after SSS rating:
How much will this food stop you from feeling hungry between meals?

Supplemental Material 5: Fatty liver index and triglyceride glucose index calculation

Calculation of Fatty Liver Index:

Some of the blood parameters will be used to calculate a Fatty Liver index (FL) using the formula of Bedogni et al [1],with measured values for BMI, fasting TG (mg/dL), fasting GGT (U/L) and waist circumference (cm), as follows:

$$FLI = (e^{0.953*\log e\ (\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\ (\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) / (1 + e^{0.953*\log e\ (\text{triglycerides}) + 0.139*\text{BMI} + 0.718*\log e\ (\text{ggt}) + 0.053*\text{waist circumference} - 15.745}) * 100$$

Calculation of Triglyceride Glucose Index:

The formula of Simental-Mendía et al.[2] will be measured with measured fasting TG (mg/dL) and fasting glucose (mg/dL), by dividing the Ln of the TG *glucose product by 2:

TyG index = Ln [(fasting triglycerides) (mg/dL) x fasting glucose (mg/dL)] / 2

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Protocol for acute and repeated dose impact of sweeteners and sweetness enhancers on appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-over, randomised, controlled trial in people with overweight/obesity. The SWEET project.

Supplemental Material 6: Trial Registration Data Set

Trial registration data	
Data category	Information
Primary registry and trial identifying number	ClinicalTrials.gov NCT04633681
Date of registration in primary registry	November 2021
Secondary identifying numbers	N/A
Source(s) of monetary or material support	European Union Horizon 2020 Program
Primary sponsor	European Union Horizon 2020 Program
Secondary sponsor(s)	N/A
Contact for public queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Contact for scientific queries	Professor Graham Finlayson (G.S.Finlayson@leeds.ac.uk)
Public title	Impact of Sweeteners on Behaviour, Physiology & Health (SWEET-WP2-P2)
Scientific title	Acute and Repeated Impact of Sweeteners and Sweetness Enhancers on Food Behaviour, Physiology & Health (SWEET Work Package 2 Phase 2)
Countries of recruitment	Denmark, France, Spain, United Kingdom
Health condition(s) or problem(s) studied	Eating Behaviour
Intervention(s)	Consumption of food product with sweetener and sweetness enhancer Consumption of sucrose-sweetened control food product
Key inclusion and exclusion criteria	Ages eligible for study: ≥ 18 ≤60 years ; Sexes eligible for study :both; Accepts healthy volunteers: yes
	Inclusion criteria: BMI 25-35kgm2; Use of contraceptive methods or not planning to become pregnant for the duration of the study (women only); Regular consumption of sugar-containing foods and willing to consume sugar and artificially-sweetened food products; Liking of the intervention foods defined by a response of 'Yes' for the product during the pre-screening interview and a score of 40% or above on the Liking Visual Analogue Scale for the sucrose-sweetened control product; Able to participate on the Clinical Investigation Days during normal working hours; Healthy as determined from the self-reported medical history or when a clinical condition exists, when this is considered to be irrelevant (i.e. not influencing study outcomes) for the study by the study medical doctor; Consuming breakfast regularly (at least 5 days per week); Able to understand and be willing to sign the informed consent form, and to follow all the study procedures and requirements; Capacity to store at-home intervention quantity of intervention product
	Exclusion criteria: Blood donation < 3 month prior to study or for full duration of the study; Food allergy, intolerance, restriction or avoidance of any of the study foods (e.g. veganism) or history of anaphylactic reaction to any food; Likelihood for disordered eating defined as a score ≥20 on the Eating Attitudes Test; Currently dieting to lose weight; Having lost or gained >4.5 kg in the last 3 months; Smoking or having quit <3 months prior to study; Habitually consuming >14 units/week of alcohol in women or >21 units/week in men in the last 3 months; Performing >10 h of intense physical activity per week in the last 3 months; Night or late shift work (ending later than 11 pm on a permanent basis). Rotational shift work allowed if can attend on days that do not follow a late/night shift; Self-reported use of drugs of abuse within the previous 12 months; Pregnancy, lactation (women only); Persons who do not have access to either (mobile) phone or internet (this is necessary when being contacted by the study personnel during the study); Insufficient communication in the national language; Proven or suspected inability, physically or mentally, to comply with the procedures required by the study protocol as evaluated by the daily study manager, site-PI, PI or clinical responsible. This includes volunteers for which insufficient collaboration may be foreseen; Subject's general condition contraindicates continuing in the study as evaluated by the daily study manager, site-PI, PI or responsible clinician; Simultaneous participation in other relevant clinical intervention studies; Previous university or college training related to eating behaviour research; Self-reported eating disorders; Diagnosed anaemia; Diagnosed diabetes mellitus; Abnormal G.I. function or structure such as malformation, angiodysplasia, active peptic ulcer; Active inflammatory bowel disease, coeliac disease, chronic pancreatitis or other disorder potentially causing malabsorption; History of G.I. surgery with permanent effect (i.e. surgical treatment of obesity); Medical history of Cardiovascular Disease (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease); Significant liver disease, e.g. cirrhosis (fatty liver disease allowed); Malignancy which is currently active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed); Thyroid diseases, except those on Levothyroxine treatment of hypothyroidism if the person has been on a stable dose for at least 3months; Psychiatric illness (e.g. major depression, bipolar disorders); Use currently or within the previous 3 months of prescription or over the counter medication that has the potential of affecting appetite, satiety or body weight incl. food supplements. Except: low dose antidepressants if they, in the judgement of the daily study manager, site-PI, PI or clinical responsible, do not affect weight or following the study protocol. Levothyroxine for

	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering medication, if the dose has changed during the last3 months (i.e., the medication is allowed if the participant has been on a stable dose for at least 3 months)
Study type	Interventional Allocation: Randomised. Double-blind, within-subjects, cross-over trial
	Primary purpose: Evaluation Phase: N/A
Date of first enrolment	April 2021
Target sample size	213
Recruitment status	Recruiting
Primary outcome(s)	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
Key secondary outcomes	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers: Glucagon-like peptide-1 (GLP-1)Incremental area under the curve for blood GLP-1 concentrations in response to each product (120 min post intake);Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product (120 min post intake).

Appendices: Informed Consent Materials and GDPR

FASt
Food Acceptance Study

Institutional logo

Informed Consent Form

Title of research project: Food Acceptance Study (FAST) [or include title in national language]

I confirm that: (please initial next to each statement to show you agree)

I have obtained written and oral information about the research project and I am informed about the aim, methods, benefits and risks of participating in the study.	
I have read and have understood the information sheet [version number] for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.	
I understand that taking part in the study involves completing a screening visit plus 6 clinical investigation days during which I will need to consume foods, provide blood [urine, and faeces] samples and fill in questionnaires.	
I understand that I will not be able to donate blood for the duration of my participation in the study.	
I understand that my participation is voluntary and that I am free to stop taking part and can withdraw from the study prior to anonymization of the data (1st March 2023) without giving any reason and without my rights being affected.	
I understand that I can ask for access to the data I provide and I can request the destruction of that data at any time prior to anonymization of the data [add date]. I understand that after anonymization of the data, I will no longer be able to request access to or withdrawal of the data I provide.	

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I understand that the data, including any identifiable data I provide will be held securely and in line with data protection requirements at [add institution].	
I understand that pseudo-anonymised data (including my participant number) will be sent to other partners within the larger EU project for testing and analysis.	
I understand that pseudo-anonymised data may also be shared with other researchers who are not working on this study for future research purposes in the public interest.	
I understand that fully anonymised data (after destruction of the ID-log) will be made available to the public (open access). As a result other external organisations or researchers will be also able to access these anonymised data for future research purposes.	
I understand that my anonymised data will be retained indefinitely on password-protected computers at [add institution].	
I consent to participate in the above study.	
[Remove if not applicable] I consent that my biological material will be stored in a research biobank at the [enter University].	
I have received a copy of this informed consent form as well as a copy of the Participant Information Sheet ([version number]) and a copy of the General Data Protection Regulation information sheet ([version number]).	

Participant name: _____

Date: _____ Signature:_____

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In case new information that has substantial influence on your health emerges from the research project, you will be informed. If you would prefer **not** to be informed about such information please mark it here _____ (insert X).

Consent from the study staff that provided the oral information:

I declare, that the participant has received both written and oral information about the research project.

I declare to the best of my knowledge and belief that the participant has received sufficient information to decide to participate in the research project.

Study staff name: _____

Date: _____ Signature: _____

National project identification: [include e.g. ethical approval number from ethical committee and date of approval]

FASt
Food Acceptance Study

Institutional logo

General Data Protection Regulation (GDPR) information for study participants

What data will be collected, how will they be used and who will see them?

Appendices: Informed Consent Materials and GDPR

In relation to your participation in the Food Acceptance STudy (FAST), a range of data will be collected from you. This document explains how your data will be used.

1. Which data will be collected and how?

The data collected includes information about health and personal data e.g. name, [civil registration number if relevant, include name/type], contact information, gender and ethnicity and biological material (i.e. blood[,urine and faeces]). The data is registered in a personal participant folder and/or in an electronic database.

Data and biological material that is sent from the intervention site to other laboratories or researchers will contain a participant number but never your name or any other personal identifying data.

The investigators and other data processors will ensure that the information collected about you is not accessed by unauthorized persons and that your identity is protected when the results of the study are published. The online questionnaire delivery platform collecting body sensations and other measures will only use basic cookies to enable the proper functioning of the program. No marketing or other tracking cookies will be used.

2. How will my data be stored?

The investigators and the data manager will take all necessary security precautions to ensure that any identifying information about you is kept confidential and stored securely in accordance with local law [include name/references] and EU Regulation.

You will be assigned a unique ID number which your data will be identified by throughout the study. All electronic forms of data will be protected with a password which can only be accessed by the researchers. All data recorded on paper will be locked in study-specific storage cabinets accessible only to the researchers on the project.

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3. How long will my data be stored for?

Pseudo-anonymised data (including your participant ID) will be stored for up to 5 years. At that point, the participant ID-log will be destroyed and the pseudo-anonymised data will become fully anonymised data.

Fully anonymised data (not including your participant ID) after completion of the study will be retained indefinitely in an open-access repository (see point 7. *Will my data be archived for use in other research projects in the future* below).

Your biological samples (i.e. blood[, urine and faeces]) will be stored temporarily at the intervention site in freezers at either -20 or -80°C and later sent to other specialist laboratories for analysis. Once the biological material has been analysed for results related to this study, it will be destroyed by the laboratory. Destruction will happen at the latest by [2025] (five years after the study has ended).

[A research biobank contains biological material that is stored for future related research. If you wish to donate any excess of your biological material from this study to the research biobank of the [add university or other identifying name]), you must state it separately below. The donation is completely voluntary and does not affect your participation in this study. Samples in the biobank of the [university name] consist of a small amount (e.g. 5 ml blood [amend as suitable]), and are stored at [include name of intervention site] in freezers at -80 ° C for a maximum of 15 years after the study has ended. In order to conduct new analyses of your biological material from this biobank in a new study, the national ethical committee must first approve the study. You can always contact the intervention site and ask to have your samples in the biobank destroyed, unless the samples have been totally anonymised beforehand, which means that no one, nor the principal investigator, can longer assign the material to you. Sample full anonymization will take place alongside full anonymisation of all other data, after 5 years from study termination at the latest.]

4. What measures are in place to protect the security and confidentiality of my data?

The site-principal investigator will store an identifier (ID)-log (“key”) that associates your participant number with your personal information. This ID-log

is stored at the intervention site separately from data and biological material in a locked room. Only a few relevant persons from the study staff have access to the ID-log including national and international authorities controlling clinical research projects e.g. the local ethical committee [and if relevant, include other local authorities]. The ID-log will be used to identify you in case it is relevant. The ID-log will be stored at the intervention site as long as it is relevant to have your contact information and for ethical and legal considerations related to the conduct of the study. The ID-log will be stored for a maximum of 5 years after the study has ended.

5. How will my data be used?

The data collected will be securely forwarded to a project data hub at the University of Navarra (Spain) and subsequently used for analysis.

In case you withdraw from the study, the data already collected from you may be used and included in the analyses if the researchers find it important for the quality of the study. Already collected data from you will therefore only be processed if it is fair and important for the study. However, you may request that your data are destroyed and no further use is made of them. Please note, it will not be possible to withdraw your data after the results have been processed (this may be approximately 3 months after the study has ended, or by the 1st of March 2023).

The results of the study, regardless of whether they are positive, negative or inconclusive, will be written-up and submitted for publication after the end of the study e.g. as a publication in a journal, a summary of the test results on the Internet or at www.clinicaltrials.gov. Published results do not contain any information that can identify you.

6. Who will have access to my data?

Your pseudo-anonymised data (including your participant number) will be securely sent to other partners within the larger EU project e.g. the University of Navarra (Spain) and the University of Surrey (UK) for analysis and your pseudo-anonymised biological material will be sent to partner laboratories, e.g. Bioiatriki S.A. (Athens, Greece) for testing and analysis. Data and biological material are

only sent from the intervention site to other laboratories with your participant number and never your name or other personal identifying data.

Pseudo-anonymised data may also be shared with other researchers who are not working on this study for future research purposes in the public interest.

After full anonymisation (destruction of the participant ID-log) the data collected in this study will be made available to the public (open access) by depositing it in an open access repository or other related archive. As a result, other external organisations or researchers will be also able to apply to access these fully anonymised data.

7. Will my data be archived for use in other research projects in the future?

Yes. We will make the fully anonymised data available to other organisations or researchers by depositing it in an open access repository or other archive. It is important that you understand that your data will be completely anonymised for these purposes, therefore there will be no way that you can be identified.

8. How will my data be destroyed?

The ID-log for all data will be stored for a maximum of 5 years after the study has ended. After that point, the ID-log will be destroyed and all data will be fully anonymized. After full anonymisation data will be available to the public (open access).

Any excess biological material will be destroyed by the handling laboratories after the analyses have been completed [keep/remove: unless you have chosen to donate some to a biobank in your local country. If this is the case, biobank material will be destroyed after 15 years following the termination of the study (i.e. by 2035)].

☐**I confirm that I have read and agree to the above information about the handling, processing and storage of my personal information in this study**

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as well as data sharing procedures between external partners within this larger EU project and other external organisations.

[Please, consider if you want to donate excess material for the biobank:

☐ **Yes**, I want to donate potential excess biological material from me to a biobank.

☐ **No**, I do not want to donate potential excess biological material from me to a biobank.]

Participant´s signature:

_____

___

Date Name

Signature

Researcher´s signature:

_____

___

Date Name

Signature

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered, name of intended registry	12
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	Supplemental Material 6
Protocol version	#3	Date and version identifier	6
Funding	#4	Sources and types of financial, material, and other support	15
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	16

Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	15
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	15
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	7 and 15
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	4
Objectives	#7	Specific objectives or hypotheses	5
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	6
Methods: Participants, interventions, and outcomes			

Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7

Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	6
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	8
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	8-9
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	9
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A: No safety/critical efficacy issues present

Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	15; Supplemental Material 4
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	N/A Data from participants who discontinue/ deviate from protocol not collected
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15; appendices
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	N/A, no analysis included
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	N/A, no analysis included

Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A: No safety/critical efficacy issues present
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A: No safety/critical efficacy issues present
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	N/A: No safety/critical efficacy issues present
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	3
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	15

Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Appendices
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	15
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	15
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A: No safety/critical efficacy issues present
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	15
Dissemination policy: authorship	#31b	Authorship eligibility guidelines and any intended use of professional writers	16
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	15
Appendices			
Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Appendices

Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12