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Protocol for a multicentre, parallel, randomised, controlled, trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in overweight adults and children. The SWEET project.

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ABSTRACT

Introduction

The aim of this randomised controlled trial (RCT) is to investigate if prolonged consumption of sweeteners and sweetness enhancers (S&SEs) within a healthy diet will improve weight loss maintenance and obesity related risk factors, and affect safety markers compared to sugar.

Methods and analysis

SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is a 1-year multicentre RCT including at least 330 adults with overweight (18-65 years, body mass index (BMI)>25 kg/m^2) and 40 children (6-12 years, BMI-for-age>85th percentile). In an initial 2-month period, adults will consume a low-energy diet with the aim to achieve ≥5% weight loss. Children are advised to consume a general healthy diet to maintain body weight, thus reducing their BMI-for-age z-score. In the following 10 months, participants will be randomised to follow a healthy *ad libitum* diet with or without S&SE products. Clinical investigations are scheduled at baseline, after 2, 6 and 12 months. The primary outcomes are body weight for efficacy and gut microbiota composition (in relation to metabolic health) for safety, both in adults. Secondary outcomes include anthropometry, risk markers for type-2-diabetes and cardiovascular diseases, questionnaires including e.g. food preferences, craving and appetite, and tests for allergenicity.

Ethics and dissemination

The trial protocol has been approved by the following national ethical committees; The research ethics committees of the capital region (Denmark), approval code: H-19040679, The medical ethics committee of the University Hospital Maastricht and Maastricht University (Netherlands), approval code: NL70977.068.19 / METC19-056s, Research Ethics Committee of the University of Navarra (Spain), approval code: 2019.146 mod1, Research Ethics Committee of Harokopio University (Greece), approval code: 1810/18-06-2019. The trial will be conducted in accordance

with the Declaration of Helsinki. Results will be published in international peer-reviewed scientific journals regardless of whether the findings are positive, negative or inconclusive.

Trial registration number: NCT04226911.

Strengths and limitations of this trial

- The trial is apparently the first of its kind to investigate long-term effects of S&SEs in the contexts of an *ad libitum* healthy diet including both foods and drinks, compared to sugar.
- It is also the first to include a 2-month weight loss period to determine the effects of S&SE foods and drinks on longer-term weight maintenance after weight loss, compared to sugar.
- A broad range of measurements related to health and safety (e.g. gut microbiota, allergenicity, specific adverse events), appetite and food preferences are included to address concerns raised in relation to S&SEs.
- This multicentre trial covers Northern, Central and Southern Europe, thereby reflecting different geographic distributions of adult and childhood obesity in Europe.
- The number of included children was reduced through the recruitment period, but this will not affect the two primary outcomes (body weight and gut microbiota composition) as sample size determination was done exclusively for adults.

INTRODUCTION

Obesity is a major global health problem giving rise to increased risk of non-communicable diseases such as type-2-diabetes (T2D) and cardiovascular diseases (CVD).[1] Sustaining energy balance is critical to maintain body weight. However, sugar contributes to the energy density of diets, and may promote a positive energy balance.[2] In 2015, the World Health Organization (WHO) strongly recommended that free sugar intake should constitute <10 energy percentage (E%) and preferably <5 E% as a conditional recommendation.[2] The latter is still not fulfilled by large parts of the population, including Denmark,[3,4] Greece,[5] Spain[6] and Netherlands.[7]

One often-recommended approach to reduce sugar intake is to replace sugar with sweeteners or sweetness enhancers (S&SEs). The use of S&SEs allows products to retain their palatability without the associated calories, creating a perception of a 'healthier' product.[8] Although drinks often constitute the largest part of S&SEs products consumed, S&SEs products also include foods. In the US and worldwide, the consumption of S&SEs products such as desserts, gums and breakfast foods has increased.[9] However, foods with S&SEs have been less extensively investigated. S&SEs compromise a variety of compounds with proposed mechanisms on health parameters,[10] however evidence is conflicting. For example, S&SEs have been claimed to result in detrimental effects on appetite, body weight, glucose metabolism, and gut microbiota.[8] In contrast, several systematic reviews and meta-analysis found no detrimental effects on appetite and body weight – rather on the contrary[11–13] and interestingly, a large 1-year study found S&SEs to be superior to water for weight loss and weight maintenance.[14] Not all reviews have come to the same conclusion, but selective citation of the different studies could be the cause.[15] In relation to postprandial glycemia and insulinemia no differences were observed between S&SEs and controls in recent systematic reviews and meta-analysis.[16,17] An aberrant gut microbiota composition and a change in composition after 7 days consumption of saccharin have been associated with impaired glucose homeostasis.[18] However, that study included only 7 human participants, conclusions were based on a post-hoc division of responders and non-responders, and there was no control group.[18]

Aim and objectives

The overall aim of the randomised controlled trial (RCT) SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is to investigate the efficacy and safety of combined (foods and drinks) and prolonged use of S&SEs - as part of a whole healthy *ad libitum* diet approach - in a population of overweight adults and children. The two primary outcomes on efficacy and safety will be assessed in adults by 1-year changes in body weight and 1-year changes in gut microbiota (in relation to metabolic health outcomes), respectively. Secondary objectives concern the effects on obesity-related risk factors such as fat mass, glucose metabolism, and lipidemia, as well as safety aspects such as allergenicity. Other outcomes include appetite sensations, food cravings and preferences and preference for sweet taste.

Hypothesis

We hypothesize that prolonged use of S&SEs in foods and drinks will result in improved body weight control due to increased palatability of the diet and thereby compliance to the recommendations for a healthy diet, compared to sugar. Further, we hypothesize that there will be no safety concerns using S&SEs in the long-term.

METHODS AND ANALYSIS

Study design

SWEET is conducted in four intervention sites; Athens, (Harokopio University of Athens, Greece), Copenhagen, (University of Copenhagen, Denmark), Maastricht, (Maastricht University, Netherlands), and Navarra (University of Navarra, Spain) covering North, Central, South and East Europe thereby reflecting different geographic distributions of obesity in Europe. In the 1-year trial both adults and families (at least 1 adult and 1 child) are included. The trial consists of an initial 2-month period followed by a 10-month randomised 2-armed parallel intervention period. For adults, the goals in these periods are first to achieve a weight loss (WL) and second to maintenance the WL. For children, the goals are first to achieve weight stability and second to maintain BMI-for-age z-score. The 10-month randomised intervention period will

be carried out by using a "fading visit" approach (Figure 1). During the trial, all participants will undergo 4 clinical investigation days (CIDs) and will be supervised by dieticians individually/familywise and/or in groups at least every 3rd month.

Originally, a 1-year follow up period was planned after the 10-month intervention period; however, it was omitted due to recruitment delay caused by the Covid-19 pandemic. Furthermore, the initial plan was to include at least one child per adult (i.e. only families). However, recruitment turned out to be very difficult and the strategy was changed to also include adults without children, because the primary outcomes and sample size determinations were based solely on adults. Screening visits were conducted between 29-Jun-2020 and 27-Sep-2021, and the last patient last visit (CID at month 12) is scheduled for 30-Sep-2022.

Patient and public involvement

Neither patients nor the public were involved in the design and conduct of the study and they will not be involved in interpretation, reporting, or dissemination of the trial.

Participants

Recruitment and screening

Participants were recruited continuously by multiple routes e.g. web-pages, social medias, newspapers, and registries (local databases or civil registration numbers). Potential adult participants were pre-screened by phone and answered questions on behalf of their child(ren). If still eligible and interested after pre-screening, they received written information and were invited to an information meeting. After the information meeting, an informed consent form and a general data protection regulation form were signed by the adult participant, and for children by the parents, or person(s) having custody and the site-PI or delegated staff. Thereafter, the screening visit was scheduled. Participants were screened in the fasting state where all in- and exclusion criteria were assessed. The recruitment has ended with inclusion of 341 adults and 38 children.

Eligibility criteria

Adults (men and women), 18-65 years, BMI≥25 kg/m^2 and children (boys and girls), 6-12 years, and BMI-for-age>85th percentile were included. Children were only included if they had an eligible adult family member (i.e. as a family) - a biological relationship was not required. However, it was required that the family lived in the same household at least 4 days/week. Participants were required to have a regular consumption of sugar-containing/sugar-sweetened products and be motivated and willing to be randomised to any of the two intervention groups. All exclusion criteria are listed in Table 1. In- and exclusion criteria are assessed at screening, however the site-PI or delegated personnel has the right to terminate participation at any time if deemed in the participant's best interest, and children are excluded if their adult family member's participation is discontinued.

Table 1: List of exclusion criteria

Adults	Children
General	
Weight change >5% 2 months prior to screening	Intensive physical training (>10 hours of per week)
Surgical treatment of obesity	Self-reported eating disorders
Blood donation <3 months prior to study initiation	Intolerance and allergies expected to interfere with the study
Change in smoking habits during the last month. (Smoking was allowed and monitored throughout the study)	Insufficient communication with national language
Regularly drinking >21 (men) or >14 (women) units of alcohol per week	Inability, physically or mental, to comply with the procedures required by the study protocol
Intensive physical training (>10 hours of per week)	Participant's general condition contraindicates continuing the study
Self-reported eating disorders	Simultaneous participation in other clinical intervention studies
Intolerance and allergies expected to interfere with the study	
Self-reported drug abuse within the previous 12 months	
Night- or shift work that ends later than 11 PM	
For women: Pregnancy, lactation	
Persons who do not have access to either (mobile) phone or Internet	
Insufficient communication with national language	
Inability, physically or mental, to comply with the procedures required by the study protocol	
Participant's general condition contraindicates continuing the study	
Simultaneous participation in other clinical intervention studies	
Medical conditions	
Diagnosed diabetes mellitus	Diagnosed diabetes mellitus
Medical history of CVD (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease)	Other diseases that may influence the study outcomes
Systolic blood pressure above 160 mmHg and/or diastolic blood pressure above 100 mmHg (measured at screening) whether on or off treatment for hypertension	
Significant liver diseases e.g. cirrhosis (fatty liver disease allowed)	
Malignancy which was active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed)	
Active inflammatory bowel disease, celiac disease, chronic pancreatitis or other disorder potentially causing malabsorption	
Thyroid diseases, except Levothyroxine treatment of hypothyroidism if not on a stable dose for at least 3 months	

Adults	Children
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 had the potential of affecting body weight incl. food supplements Exceptions related to medical conditions: I) Cholesterol or blood pressure lowering medication were allowed if the participant's dose had not changed during the last 3 months II) low dose antidepressants if they, in the judgement of the investigator, did not affect weight or study participation. III) Levothyroxine for treatment of hypothyroidism if on a stable dose for at least 3 months	Use currently or within the previous 3 months of prescription or over the counter medication that had the potential of affecting body weight incl. food supplements
Laboratory screening[1]	
Glucose >7.0 mmol/L	-
Haemoglobin: women; <7.5 mmol/L (Copenhagen, Maastricht, Navarra, Athens) men; <8.5 mmol/L (Copenhagen, Maastricht) and <8.1 mmol/L (Navarra, Athens)	
For Maastricht participants only: Creatinine <50 µmol/L and >100 µmol/L ALT >34 IU	

[1]Fasting blood sample was collected from adults and locally analysed to assess glucose and haemoglobin levels, and some additional values at Maastricht.
ALT, Alanine transaminase; CVD, cardiovascular diseases; IU, international unit

Randomisation

After screening, eligible participants were randomly assigned to one of the two intervention groups in a 1:1 ratio by a site-specific randomisation list created by a person in Copenhagen not involved in the trial. The randomisation was stratified by gender, age (<40 years or ≥40 years) and BMI (<30 or ≥30 kg/m^2), and stratification was implemented by sequentially assigning families and adults from each stratum to the two interventions in blocks of 4, using the software R. Each household was randomised to the same intervention determined by the oldest member of the household. Although randomisation was done after screening, it is not revealed to the household/participant before completion of the initial 2-month period.

Intervention

This 1-year trial is divided into two periods of 2- and 10-months duration (Figure 1) with the second period being the randomised intervention period.

Two-month period

In the initial 2-month period, adults - regardless of randomisation - receive a low-energy diet (LED) (Cambridge Weight Plan, Northants, United Kingdom). If the WL criteria of ≥5% is not

achieved, the participant will be excluded. During the 2-month period, adults visit the intervention site 2-3 times for collection of LED products, weighing and dietetic counselling. The LED consists of 3,347-4,184 kJ/d, 15-20 E% fat, 35-40 E% protein and 45-50 E% carbohydrate. Four products per day will be provided as shakes, soups, ready-to-drink products and bars. Additionally, 200 g tomatoes, 125 g cucumber, 50 g lettuce and chewing of maximum 6 pieces of sugar-free chewing gum or pastilles per day are allowed. For some adults (e.g. BMI >40 kg/m^2 or achieving a BMI ≤23 kg/m^2 during the LED without a wish to lose more weight), the LED may be supplemented with milk/yoghurt, but only if it is expected that the required 5% WL can be achieved.

In the initial 2-month period, children are encouraged to follow the dietary recommendations of the American Academy of Paediatrics on the prevention, assessment and treatment of overweight and obesity.[19] The goal is to obtain weight stability, which will reduce BMI-for-age z-score. Children are welcome to visit the intervention site for weighing and dietician counselling, however it is not mandatory.

Ten-month period with S&SEs and sugar diets

During the 10-month randomised intervention period, dietary counselling sessions will be practiced as individual (i.e. household) counselling sessions at months 2 and 6 and when Covid-19 restrictions allow in intervention groups (months 4 and 9). Otherwise, individual counselling sessions will be scheduled. The goals are to maintain WL for adults and BMI-for-age z-score for children. Further reduction in weight or BMI-for-age z-score is allowed, if the participant is compliant with the intervention, but counselling sessions will only cover maintenance aspects.

The two intervention diets are I) a healthy diet with <10 E% sugar allowing foods and drinks with S&SEs (S&SEs group) and II) a healthy diet with <10 E% sugar not allowing foods and drinks with S&SEs (Sugar group). Both diets are *ad libitum.* To secure dietary adherence calculation of maximum sugar intake (g) will be based on a diet with 9.5 E% sugar. The maximum allowed sugar intake will be calculated individually based on body weight at month 2 (re-calculated at month 6), using the formula by Henry [20] multiplied by the physical activity

level (PAL). A unit system for the sugar and S&SEs intake has been developed, where individual maximum sugar intake is converted to a certain number of units per day (and week) (1 unit corresponding to 10 g sugar). One unit of S&SE product in the S&SE group is equal to the amount - in weight or volume - of one unit sugar-rich product in the Sugar group. For the S&SE group, as many sugar-containing products as possible should be replaced by S&SE products. Food exchange lists, covering categories listed in Table 2 including pictures of products, amounts and units per product, guide the participants in the two groups. Additional details and examples of the two interventions are provided in Table 3. Due to the characteristics of the study, blinding is not possible, however all effort to blind study staff taking measurements and persons doing statistical analysis will be done.

Table 2: Foods and drinks relevant for the 10-month randomized intervention period

Category	Examples
Drinks	Carbonated soft drinks, fruit juice, non-carbonated soft drinks, cocoa powder, mixture of fruit syrup and water, energy drinks, pre-packed juices and nectars, protein shakes, energy drinks
Milk products	Flavoured yoghurts, yoghurt drinks, milk shakes, chocolate milk, fermented milk, cold butter milk
Breakfast cereals	Breakfast cereals, muesli, cereals bars, rolled oats
Sugar, honey and marmalade	Sugar, syrup, honey, marmalade, jam, compote
Chocolate and bars	Chocolate with and without filling, chocolate bars, chocolate/hazelnut paste/spread, thin sliced chocolate
Desserts	Pudding, mousse, cold soufflé, custard, strained stewed fruit, Greek jelly, pancakes
Ice cream	Ice cream, sorbet, ice lolly
Candy	Wine gum, liquorice, Bon-bon mix, marshmallow, marzipan
Cake and biscuits	Cake, cookies, biscuits, Danish pastry, sponge cake

Table 3: Description of diets in the 10-month randomized intervention period

	Sugar group	S&SE group
Sugar-containing products	<10 E% added sugar.	<10 E% added sugar and as little as possible.
S&SE products	Not allowed. Except for up to 2 pieces of sugar-free chewing gum per day.	Allowed. And without any restrictions on specific types of S&SEs.
Units	Consumption of a maximum number of units (corresponding to 9.5 E% added sugar) of sugar-containing products each day/week.	Unit calculation (corresponding to 9.5 E% added sugar in weight/volume) will guide the participant to ensure intake of <10 E% added sugar. As many sugar-containing products in the diet as possible should be replaced with S&SE-containing products. Ideally, the amount of S&SEs products corresponding to the maximal units from sugar-containing products should be consumed. However, if a participant experiences AE, they are recommended to consume

	Sugar group	S&SE group
		less than the calculated units and change to other S&SE products (e.g. avoid sugar-alcohols).
Example	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. 5 units per day or 35 units per week is then the maximum allowed intake of added sugar for this participant.	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. Ideally, this participant should consume 5 units per day or 35 units per week of S&SE containing products. One unit is equivalent to 1 unit in the sugar group (in weight or volume).

E%, energy percentage; S&SEs, sweeteners and sweetness enhancers

Compliance:

Participants are required to record intake of all foods and drinks (pen and paper) for 4 days (3 weekdays and 1 weekend day) at months 0 and 12 with information on time, type/brand names, cooking and processing methods, weight or household measures. Daily average intake of energy, macro- and micro-nutrients will be calculated by national nutritional software in the 4 intervention sites. Furthermore, intake of sugar and S&SEs in units is assessed. Additionally, adults complete a food frequency questionnaire about sweet products (sFFQ), and from a 24-hour urine collection, biomarkers of S&SEs will be analysed by Wageningen University, Netherlands, and excretion of urea/nitrogen will be analysed locally.

Data collection and outcomes

Including information meeting, screening, counselling sessions and CIDs, the trial consists of a minimum of 10 and 6 visits for adults and children, respectively. Data is collected according to standard operation procedures (SOPs) and Table 4 shows activities/data collection at each visit. Most data will be collected at months 0, 2, 6 and 12 where participants have fasted for a minimum of 10 hours, and avoided intensive physical exercise, coffee and smoking for 12 hours prior to the CIDs.

Table 4: Flow chart for adults (A) and children (C) (full sampling at months 0 and 12)

	Pre-screening	**Information meeting**	**Screening**	**Baseline**		***2-month period (CID1-CID2)***		***10-month randomised intervention period (CID2-CID4)***			**1-year assessment**
CID	**-**	**-**	**-**	**CID1**	**-**	**-**	**CID2**	**-**	**CID3**	**-**	**CID4**
Visit	**-**	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**
Month	**-**		**-**	**0**	**0.5**	**1**	**2**	**4**	**6**	**9**	**12**
Inclusion/exclusion criteria	A+C		A+C								
Signing Informed Consent		A+C[0]									
Med. hist., medication etc.			A+C								
Randomisation of the oldest family/household member			A								
Supervision/counselling				**A+C[1]**		A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**
Collection of LED products				**A[#]**	A[#]	A[#]	**A[#]**				
Body weight and height[4]			A+C	**A+C**	A[3]	A[3]	**A+C**	A[3]	**A+C**	A[3]	**A+C**
Waist and hip circumference				**A+C**			**A+C**		**A+C**		**A+C**
Body composition				**A+C[5]**			**A**				**A+C[5]**
Blood pressure and heart rate			A+C	**A+C**			**A+C**		**A+C**		**A+C**
Fasting blood samples			A[6]	**A+C**			**A+C[7]**		**A+C**		**A+C**
Adverse events and concomitant medication				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity (skin prick test)				**A**							**A**
24h urine collection (content of S&SEs)				**A**					**A**		**A**
Faecal spot sample				**A**			**A**		**A**		**A**
4-day dietary record				**A+C**							**A+C**
Questionnaires (electronic platforms):											
General background questionnaire				**A+C**							
Physical activity				**A+C**							**A+C**
Three factor eating questionnaire				**A+C**							**A+C**
Leeds food preference questionnaire				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity				**A+C**							**A+C**
Craving for sweet taste				**A**							**A**
Perception of S&SEs				**A**					**A[8]**		**A[8]**
Control of eating				**A**			**A**		**A**		**A**
Subjective appetite sensations				**A**			**A**		**A**		**A**
Sweet food frequency questionnaire (FFQ)				**A**							**A**
Diet satisfaction				**A**					**A**		**A**
Perception and evaluation of the intervention									**A**		**A**
Quality of life				**A**							**A**
Puberty				**C**					**C**		**C**

[#]Adults will collect LED products from the intervention site every 2nd or 3rd week during the 2-month period. At months 0.5 and 1.5 (optional) the adults will be weighed and have the opportunity to consult a dietician.
[0]For children, the informed consent is signed by the parents/guardians.
[1]Individual/family counselling is preferably scheduled at the same day as the CID.
[2]Group counselling, children participation is preferred, but not mandatory.
[3]Fasting is not required for this body weight measurement.
[4]Height is only measured at screening for adults.
[5]At University of Maastricht, body composition is not measured in children.
[6]At screening, fasting blood samples will be analysed at each intervention site. All other blood samples are analysed at the Central Laboratory (Bioiatriki).
[7]At University of Maastricht, a fasting blood sample is not drawn from children.
[8]A shorter version of the questionnaire is used at CID3-4.
A: adult. C: child. CID: clinical investigation day. LED: low-energy diet. S&SEs: sweeteners and sweetness enhancers.

Primary outcomes

This trial has two independent primary outcomes. The primary outcome for efficacy is 1-year change in body weight. The primary outcome for safety is 1-year change in gut microbiota composition associated with impaired health (e.g. change in microbial beta-diversity and composition). Both outcomes relate only to adults, and hence the required sample size was calculated for adults only.

Body weight

Body weight is measured to the nearest 0.1 kg using a digital scale with the participant wearing underwear/light clothes. Fasting body weight will be measured at screening and CIDs, however fasting is not required, when body weight is measured at other visits.

Gut microbiota

Gut microbiota composition will be assessed from faecal spot samples collected at home prior to all CIDs. Samples are immediately frozen (-20 °C) and later transported to the intervention site in cooling bags, whereafter they are stored at -80°C. Samples will be analysed targeting the V3-V4 regions of 16s rRNA genes by Illumina sequencing at Maastricht University, Netherlands.

Secondary outcomes

Secondary outcomes include changes in anthropometry and body composition (for children, BMI-for-age z-score), risk factors for T2D and CVD, allergenicity, adverse events (AE), and concomitant medication. Additionally, some secondary outcomes will be assessed in adult sub-groups e.g. gut-brain signalling markers, postprandial energy expenditure and substrate oxidation, physical activity, liver fat, adipose tissue and lipid metabolism, brain reward, insulin sensitivity markers, composition and functionality of the human gut microbiota in vitro. Furthermore, children's gut microbiota composition may be analysed depending on the final sample size.

Anthropometry

Height is measured to the nearest 0.5 cm using a stadiometer at screening and for children at all

CIDs. For adults, BMI is calculated as body weight (kg) / height^2 (m^2). For children, the WHO AnthroPlus software (www.who.int/tools/growth-reference-data-for-5to19-years/application-tools) is applied to calculate BMI-for-age percentile and z-score. Waist and hip circumferences are measured twice with a non-elastic tape measure on the skin to the nearest 0.5 cm, and the average is calculated. Waist circumference is measured halfway between the lowest rib and iliac crest during exhalation. Hip circumference is measured as the largest circumference in the area around the buttock. Dual-energy X-ray absorptiometry (DXA) scans are performed in underwear to assess body composition including fat percentage, fat mass and fat free mass.

Blood pressure and heart rate

After minimum 5 minutes rest in a sitting position, blood pressure (mmHg) and heart rate (beats per minute) are measured three times on the right arm with an automatically inflated cuff. An average is calculated from the last two measurements when the two measurements differ with ≤5 mmHg. If either the systolic or diastolic blood pressure differ by >5 mmHg, a fourth measurement is performed and the average calculated from the third and fourth measurement.

Blood samples

Fasting venous blood samples are drawn at all CIDs, except at month 2 for children at Maastricht due to Ethical concerns. Serum samples are collected for analyses of lipids (triglycerides, total, low-density lipoprotein and high-density lipoprotein cholesterol), alanine aminotransferase, aspartate aminotransferase, insulin, C-reactive protein and immunoglobulin E. Plasma is collected for glucose analysis and full blood for HbA1c analysis. All samples are stored locally at -80 ºC until shipment to the central lab at Bioiatriki S.A., Greece.

Skin prick test

For adults, a skin prick test is performed on the forearm. One drop of the allergens hazel, alder, birch, grass mix, artemisia absinthium, ragweed, alternaria, moulds mix, cat, dog, dermatophagoides pteronyssinus and dermatophagoides mix as well as positive and negative control solutions are applied. The response is recorded after15 minutes.

Adverse events and concomitant medication

All AEs experienced after inclusion and during the trial are registered. At CIDs, the participant is asked if he/she has noticed any unfavourable events since the last CID. During the 10-month randomised intervention period, participants - regardless of intervention - are asked directly about certain AEs that may be related to consumption of S&SE i.e. gastrointestinal symptoms and headache. All medication necessary for the participants' health and which is not in the protocol exclusion criteria may be continued during the trial. At CIDs, the participant is asked if he/she has taken any new medicine or has changed dosage of already registered medicine.

Other outcomes

Questionnaires are used to obtain information about sociodemographic characteristics such as education, occupation, household income etc., physical activity, quality of life, and to investigate subjective neuro-behavioural indices e.g. food preferences and preference for sweet taste, perception of S&SEs, cravings, subjective appetite sensations, and perception and evaluation of the 10-month randomised intervention period. Furthermore, puberty is assessed for children. All questionnaires are prepared in English and later translated into local language. The majority of questionnaires will be delivered by a Questionnaire Delivery Platform (QDP) implemented by NetUnion, Switzerland. At baseline all questionnaires are completed at the intervention sites, but before other CIDs adults can complete those delivered by the QDP at home prior to the CID. Children always complete all questionnaires at the intervention site. Two questionnaires are always completed at the intervention site; one about perception of S&SEs (delivered by the Qualtrics platform via a weblink) and one about food rewards assessed by the Leeds Food Preference Questionnaire (E-prime software).

Statistical methods

Sample size determination

The sample size calculation is based on adults for the two primary outcomes. For body weight, a clinically meaningful effect of 1.5 kg placebo subtracted body weight has previously been

approved by the European Food Safety Authority.[21] Based on a similar trial [22] we estimated that a difference of 1.5 kg with a SD of ± 3.5 kg, a 90% power, a two-sided α of 0.05 and an estimated drop-out of 30% would require inclusion of minimum 330 adult participants. For change in gut microbiota, a ± 10% change in 20 of the 50 most abundant operational taxonomic units (OTUs) with an alpha of <0.05%, would require 100 complete samples. The inclusion of at least 330 adults (approximately 25% of the participants per intervention site) is therefore also sufficient to detect possible changes in gut microbiota.

Statistical analysis plan

As part of the SWEET project a statistical analysis plan has been developed. For body weight, 1-year change between the two interventions will be analysed by analysis of covariance (ANCOVA) linear mixed model, with change in body weight as response; treatment group and relevant covariates e.g. age, gender and BMI, are fixed effects, and participant ID and intervention site are random (intercept) effects. Intention-to-treat principle will be applied on those completing the initial 2-month period. Additionally, complete-case analyses (all dropouts omitted) and per protocol analyses (only compliant participants) as well as analyses including additional covariate adjustments and intermediate time points will be applied.

For gut microbiota, 1-year change in microbial diversity and microbial composition (relative abundance at phyla and genera level) will be analysed. Paired Wilcoxon test is used to study within intervention changes in relative abundance, and linear mixed models with Benjamin-Hochberg correction for multiple testing will be used for between intervention comparisons.

Data will be presented with the use of standard descriptive statistics shown as mean (SD) or median (Q1:Q3) for normally and non-normally distributed data, respectively, and categorical data by percentages. Results will be presented as mean difference in changes ± SEM or 95% CIs and p-values when relevant. A statistical level of 0.05 will be applied and graphical models will be carried out to assess model assumptions. When relevant, transformation e.g. logarithm will be applied or non-parametric statistical tests will be performed.

For secondary outcomes on continuous data, the main analysis will compare the 1-year mean change between the two treatment groups by use of the ANCOVA-type linear mixed model defined above without any multiplicity adjustment or imputation of missing values (i.e. available-case analyses). Additional sensitivity analyses may be carried out as appropriate in the same way as for the primary outcome. Furthermore, analysis of repeated measures will be performed using linear mixed models including time×treatment interaction, time, and treatment effects, covariates (e.g. age, gender, BMI) as fixed effects, and participant ID and intervention sites as random

effect. In case of significant time×treatment interaction, differences between treatments will be identified per time point. Mean changes will be compared between the groups using the estimated mean difference and approximate t-tests derived from the fitted linear mixed models (assuming a two-sided alternative). For secondary categorical outcome e.g. (yes/no, 0/1/2, etc.) logistic or ordinal mixed effects model including the same fixed and random effects as the linear mixed models will be used.

ETHICS AND DISSEMINATION

The trial will be conducted in accordance with the Declaration of Helsinki[23] and this master protocol (version 3.0, 28-Oct-2020) is approved by the responsible national/regional committee in the 4 countries from where consent to all previously and future amendments to the protocol was and will be obtained. All adults receive the LED products free of charge. At Copenhagen, Navarra and Athens participants will not receive reimbursement for their participation. At Maastricht, travel expenses and financial compensation are provided to all eligible participants (125 Euros for adults without child(ren) and 250 Euros for one adult and one child + 80 Euros per extra family member).

There are no risks related to the dietary interventions, however discomfort may occur. The LED (not provided for children) contains all needed nutrients, but only little energy and therefore adults may experience headaches, dizziness, tiredness and nausea particularly in the first few days. Constipation, stomach cramps or more profound nausea can occur and information on this is given before inclusion, however allergic reactions to the LED, are rare. The sugar and S&SEs intervention products are commercially available foods and drinks purchased in the supermarket and no adverse side effects are expected. However, changes in gastrointestinal symptoms e.g. bloating and excess gas production may occur depending on the participant's habitual intakes of fibre and types of S&SEs e.g. sugar alcohols. At each intervention site a physician can be consulted in case of medical uncertainties.

Some study procedures involve risks, however, the procedures implemented are designed to minimize these. Drawing blood samples will seldom cause harm besides that associated with the insertion, however children will be offered local anaesthetic Emla patches to reduce pain. A maximum of 80 ml and 125 ml of blood is drawn during the 1-year trial for children and adults, respectively. For children, this is less than 1 ml blood/kg body weight per donation which is considered safe. Fertile women will be tested for pregnancy before DXA scanning and excluded from the trial if pregnant. The DXA scans will induce minor radiation (<0.010 mSv per scan). Scanning will be done 2 and 3 times during the 1-year trial for children and adults, respectively, and only one re-scan will be allowed per CID. The skin prick test, only performed in adults, is anticipated to cause very little discomfort. A positive reaction, may cause itching, which will be treated with a salve. In very rare cases a systemic anaphylactic reaction can occur and emergency equipment is in place.

For children, special attention is given to ensure that the child is not forced to participate by the adult family member. Furthermore, a child cannot remain included if the adult family member drops out or is excluded from the trial.

All participants will be insured against injury caused by their participation according to local legal requirements. The trial is monitored by European clinical research infrastructure network (ECRIN) to ensure compliance with the protocol and SOPs. All trial-related information will be recorded, handled and stored safely allowing accurate reporting, interpretation and verification. All data will be collected in a central DataHub at Copenhagen from where pseudo-anonymised data can be requested before 2032 via a data sharing contract. From 2032 fully anonymised data can be transferred. Source data is collected on paper first or is entered directly into the electronic systems e.g. the QDP, the Qualtrics platform and/or the Research Electronic Data Capture (REDCap) tool hosted at University of Copenhagen.[24,25] REDCap is a secure, web-based software platform designed to support data capture. Source data from DXA scans and analysis of biological material are registered on the device or related hardware, whereas the source data from

dietary records (handwritten on paper) will be entered into a national software program for analysis. The sponsor/investigator will provide direct access to source data/documents for inspection.

Results will be published in international peer-reviewed scientific journals regardless of whether the findings are positive, negative or inconclusive.

AUTHORS' CONTRIBUTIONS

The SWEET EU-project was initiated by JCGH, AR, and JH. The protocol for the SWEET intervention trial was written by LK, AR and YM, LK, AR, YM, EEB and JAM contributed to the design of the trial. AR, YM, EEB, and JAM are principal investigators (PI) at the 4 intervention sites. LK and AR drafted the manuscript and YM, EEB and JAM critically reviewed the manuscript. All authors read and approved the final manuscript.

FUNDING STATEMENT

PI, AR, is also the sponsor (e-mail: ara@nexs.ku.dk, Phone: +45 21 30 69 12, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark). The trial is funded by the Horizon2020 program: *Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability* (acronym: SWEET, grant # 774293) and funding covers salary for project personal, supplies, remuneration, and dissemination of results. The amount is deposited in a project account subject to audits/public revision.

COMPETING INTERESTS STATEMENT

AR has received honoraria from Unilever, Nordic Sugar, and the International Sweeteners Association. CEH's research centre provides consultancy to, and has received travel funds to present research results from organisations supported by food and drink companies. JCGH and JH have received project funds from the American Beverage Association. TL works for a company, NetUnion sarl, which has no conflict of interest in the study outcome.

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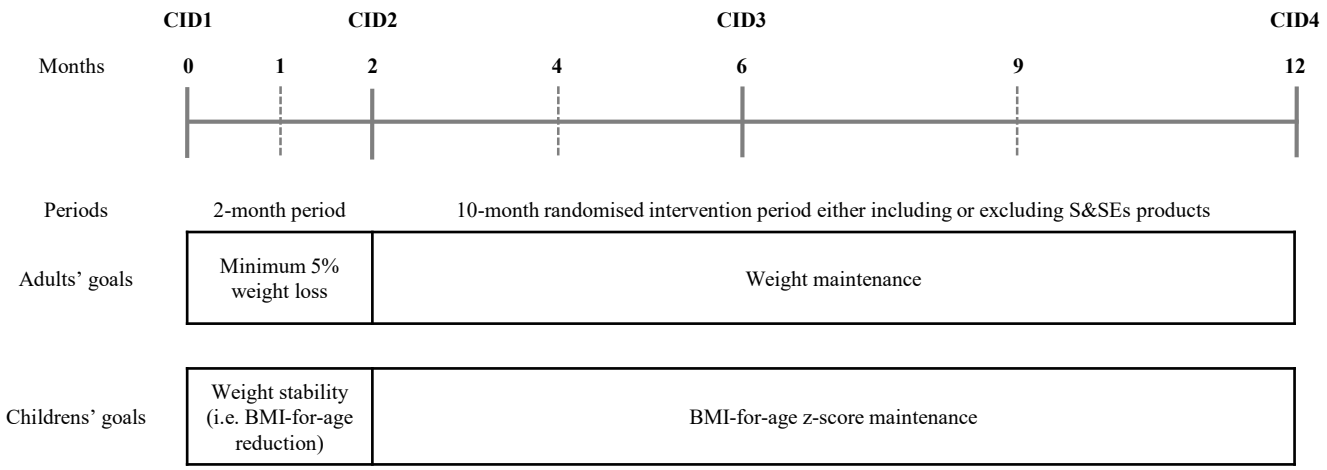
FIGURE LEGEND

Figure 1: Overall study design.
Solid lines are CIDs and dashed lines are dietary counselling sessions where non-fasting body weight of adults is measured. Additionally, LED products for adults will be collected from the intervention site every 2nd or 3rd week during the initial 2-month period.
BMI: body mass index. CID: clinical investigation day; S&SEs, sweeteners and sweetness enhancers.


CID1
CID2
CID3
CID4
Months
0
1
2
4
6
9
12
Periods
2-month period
10-month randomised intervention period either including or excluding S&SEs products
Adults' goals
Minimum 5% weight loss
Weight maintenance
Childrens' goals
Weight stability (i.e. BMI-for-age reduction)
BMI-for-age z-score maintenance

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2+21
	5b	Name and contact information for the trial sponsor	21
	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	21
	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)	21
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	5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6

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, noninferiority, exploratory)	6-7+9
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	6
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)	7-8+ Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11 + Table 2+3
	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)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
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	14-17
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)	Figure 1 +Table 4
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	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
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	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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	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	17
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	20
Statistical methods	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	17-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17-18
	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)	18

Methods: Monitoring			
Data monitoring	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	20
	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	NR
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	16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NR
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)	18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NR
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	19-20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
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	18-19

Dissemination policy	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	4+20
	31b	Authorship eligibility guidelines and any intended use of professional writers	NR
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	The master version can be delivered by request
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	NR

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Protocol for a multicentre, parallel, randomised, controlled, trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in overweight adults and children. The SWEET project.

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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Evidence based practice, Diabetes and endocrinology, Public health

Keywords:	Nutrition < TROPICAL MEDICINE, DIABETES & ENDOCRINOLOGY, Microbiology < NATURAL SCIENCE DISCIPLINES, MICROBIOLOGY, Allergy < THORACIC MEDICINE

Protocol for a multicentre, parallel, randomised, controlled, trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in overweight adults and children. The SWEET project.

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Word count: 4000

Keywords: sugar, body weight, gut microbiota, weight loss, weight maintenance, weight loss maintenance, obesity, anthropometry, type 2 diabetes, cardiovascular diseases, allergenicity.

ABSTRACT

Introduction

The aim of this randomised controlled trial (RCT) is to investigate if prolonged consumption of sweeteners and sweetness enhancers (S&SEs) within a healthy diet will improve weight loss maintenance and obesity related risk factors, and affect safety markers compared to sugar.

Methods and analysis

SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is a 1-year multicentre RCT including at least 330 adults with overweight (18-65 years, body mass index (BMI)>25 kg/m^2) and 40 children (6-12 years, BMI-for-age>85th percentile). In an initial 2-month period, adults will consume a low-energy diet with the aim to achieve ≥5% weight loss. Children are advised to consume a general healthy diet to maintain body weight, thus reducing their BMI-for-age z-score. In the following 10 months, participants will be randomised to follow a healthy *ad libitum* diet with or without S&SE products. Clinical investigations are scheduled at baseline, after 2, 6 and 12 months. The primary outcomes are body weight for efficacy and gut microbiota composition (in relation to metabolic health) for safety, both in adults. Secondary outcomes include anthropometry, risk markers for type-2-diabetes and cardiovascular diseases, questionnaires including e.g. food preferences, craving and appetite, and tests for allergenicity.

Ethics and dissemination

The trial protocol has been approved by the following national ethical committees; The research ethics committees of the capital region (Denmark), approval code: H-19040679, The medical ethics committee of the University Hospital Maastricht and Maastricht University (Netherlands), approval code: NL70977.068.19 / METC19-056s, Research Ethics Committee of the University of Navarra (Spain), approval code: 2019.146 mod1, Research Ethics Committee of Harokopio University (Greece), approval code: 1810/18-06-2019. The trial will be conducted in accordance

with the Declaration of Helsinki. Results will be published in international peer-reviewed scientific journals regardless of whether the findings are positive, negative or inconclusive.

Trial registration number: NCT04226911.

Strengths and limitations of this trial

- The trial investigates long-term effects of S&SEs in the contexts of an *ad libitum* healthy diet including both foods and drinks, compared to sugar.
- It includes a 2-month weight loss period to determine the effects of S&SE foods and drinks on longer-term weight maintenance after weight loss, compared to sugar.
- A broad range of measurements related to health and safety, appetite and food preferences are included to address concerns raised in relation to consumption of S&SEs.
- This multicentre trial covers Northern, Central and Southern Europe, thereby reflecting different geographic distributions of adult and childhood obesity in Europe.
- A potential limitation is that the number of included children was reduced through the recruitment period, but this will not affect the two primary outcomes as sample size determination was done exclusively for adults.

INTRODUCTION

Obesity is a major global health problem giving rise to increased risk of non-communicable diseases such as type-2-diabetes (T2D) and cardiovascular diseases (CVD).[1] Sustaining energy balance is critical to maintain body weight. However, sugar contributes to the energy density of diets, and may promote a positive energy balance.[2] In 2015, the World Health Organization (WHO) strongly recommended that free sugar intake should constitute <10 energy percentage (E%) and preferably <5 E% as a conditional recommendation.[2] The latter is still not fulfilled by large parts of the population, including Denmark,[3,4] Greece,[5] Spain[6] and Netherlands.[7]

One often-recommended approach to reduce sugar intake is to replace sugar with sweeteners or sweetness enhancers (S&SEs). The use of S&SEs allows products to retain their palatability without the associated calories, creating a perception of a 'healthier' product.[8] Although drinks often constitute the largest part of S&SEs products consumed, S&SEs products also include foods. In the US and worldwide, the consumption of S&SEs products such as desserts, gums and breakfast foods has increased.[9] However, foods with S&SEs have been less extensively investigated. S&SEs compromise a variety of compounds with proposed mechanisms on health parameters,[10] however evidence is conflicting. For example, S&SEs have been claimed to result in detrimental effects on appetite, body weight, glucose metabolism, and gut microbiota.[8] In contrast, several systematic reviews and meta-analysis found no detrimental effects on appetite and body weight – rather on the contrary[11–13] and interestingly, a large 1-year study found S&SEs to be superior to water for weight loss and weight maintenance.[14] Not all reviews have come to the same conclusion, but selective citation of the different studies could be the cause.[15] In relation to postprandial glycemia and insulinemia no differences were observed between S&SEs and controls in recent systematic reviews and meta-analysis.[16,17] Following consumption of S&SEs changes in the gut microbiota composition and functionality have been debated as a food safety issues because some changes in specific bacteria have been associated with diseases and risk markers of diseases.[18,19]. As an example, a change in microbial composition after 7 days consumption of saccharin has been associated with impaired glucose

homeostasis.[20] However, that study included only 7 human participants, conclusions were based on a post-hoc division of responders and non-responders, and there was no control group.[20] In general, safety concerns have been derived from animal studies using S&SE doses far above habitual intake in humans and controlled long-term human intervention studies are warranted.[19]

Aim and objectives

The overall aim of the randomised controlled trial (RCT) SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is to investigate the efficacy and safety of combined (foods and drinks) and prolonged use of S&SEs - as part of a whole healthy *ad libitum* diet approach - in a population of overweight adults and children. The two primary outcomes on efficacy and safety will be assessed in adults by 1-year changes in body weight and 1-year changes in gut microbiota (in relation to metabolic health outcomes), respectively. Secondary objectives concern the effects on obesity-related risk factors such as fat mass, glucose metabolism, and lipidemia, as well as safety aspects such as allergenicity. Other outcomes include appetite sensations, food cravings and preferences and preference for sweet taste.

Hypothesis

We hypothesize that prolonged use of S&SEs in foods and drinks will result in improved body weight control due to increased palatability of the diet and thereby compliance to the recommendations for a healthy diet, compared to sugar. Further, we hypothesize that there will be no safety concerns using S&SEs in the long-term.

METHODS AND ANALYSIS

Study design

SWEET is conducted in four intervention sites; Athens, (Harokopio University of Athens, Greece), Copenhagen, (University of Copenhagen, Denmark), Maastricht, (Maastricht University, Netherlands), and Navarra (University of Navarra, Spain) covering North, Central,

South and East Europe thereby reflecting different geographic distributions of obesity in Europe. In the 1-year trial both adults and families (at least 1 adult and 1 child) are included. The trial consists of an initial 2-month period followed by a 10-month randomised 2-armed parallel intervention period. For adults, the goals in these periods are first to achieve a weight loss (WL) and second to maintain the WL. For children, the goals are first to achieve weight stability and second to maintain BMI-for-age z-score. The 10-month randomised intervention period will be carried out by using a "fading visit" approach (Figure 1). During the trial, all participants will undergo 4 clinical investigation days (CIDs) and will be supervised by dieticians individually/familywise and/or in groups at least every 3rd month.

Originally, a 1-year follow up period was planned after the 10-month intervention period; however, it was omitted due to recruitment delay caused by the Covid-19 pandemic. Furthermore, the initial plan was to include at least one child per adult (i.e. only families). However, recruitment turned out to be very difficult and the strategy was changed to also include adults without children, because the primary outcomes and sample size determinations were based solely on adults. Screening visits were conducted between 29-Jun-2020 and 27-Sep-2021, and the last patient last visit (CID at month 12) is scheduled for 30-Sep-2022.

Patient and public involvement

Neither patients nor the public were involved in the design and conduct of the study and they will not be involved in interpretation, reporting, or dissemination of the trial.

Participants

Recruitment and screening

Participants were recruited continuously by multiple routes e.g. web-pages, social medias, newspapers, and registries (local databases or civil registration numbers). Potential adult participants were pre-screened by phone and answered questions on behalf of their child(ren). If still eligible and interested after pre-screening, they received written information and were invited to an information meeting. After the information meeting, an informed consent form and

a general data protection regulation form were signed by the adult participant, and for children by the parents, or person(s) having custody and the site-PI or delegated staff. Thereafter, the screening visit was scheduled. Participants were screened in the fasting state where all in- and exclusion criteria were assessed. The recruitment has ended with inclusion of 341 adults and 38 children.

Eligibility criteria

Adults (men and women), 18-65 years, BMI≥25 kg/m^2 and children (boys and girls), 6-12 years, and BMI-for-age>85th percentile were included. Children were only included if they had an eligible adult family member (i.e. as a family) - a biological relationship was not required. However, it was required that the family lived in the same household at least 4 days/week. Participants were required to have a regular consumption of sugar-containing/sugar-sweetened products and be motivated and willing to be randomised to any of the two intervention groups. All exclusion criteria are listed in Table 1. In- and exclusion criteria are assessed at screening, however the site-PI or delegated personnel has the right to terminate participation at any time if deemed in the participant's best interest, and children are excluded if their adult family member's participation is discontinued.

Table 1: List of exclusion criteria

Adults	Children
General	
Weight change >5% 2 months prior to screening	Intensive physical training (>10 hours of per week)
Surgical treatment of obesity	Self-reported eating disorders
Blood donation <3 months prior to study initiation	Intolerance and allergies expected to interfere with the study
Change in smoking habits during the last month. (Smoking was allowed and monitored throughout the study)	Insufficient communication with national language
Regularly drinking >21 (men) or >14 (women) units of alcohol per week	Inability, physically or mental, to comply with the procedures required by the study protocol
Intensive physical training (>10 hours of per week)	Participant's general condition contraindicates continuing the study
Self-reported eating disorders	Simultaneous participation in other clinical intervention studies
Intolerance and allergies expected to interfere with the study	
Self-reported drug abuse within the previous 12 months	
Night- or shift work that ends later than 11 PM	
For women: Pregnancy, lactation	
Persons who do not have access to either (mobile) phone or Internet	
Insufficient communication with national language	
Inability, physically or mental, to comply with the procedures required by the study protocol	
Participant's general condition contraindicates continuing the study	
Simultaneous participation in other clinical intervention studies	
Medical conditions	

Adults	Children
Diagnosed diabetes mellitus	Diagnosed diabetes mellitus
Medical history of CVD (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease)	Other diseases that may influence the study outcomes
Systolic blood pressure above 160 mmHg and/or diastolic blood pressure above 100 mmHg (measured at screening) whether on or off treatment for hypertension	
Significant liver diseases e.g. cirrhosis (fatty liver disease allowed)	
Malignancy which was active or in remission for less than five years after last treatment (local basal and squamous cell skin cancer allowed)	
Active inflammatory bowel disease, celiac disease, chronic pancreatitis or other disorder potentially causing malabsorption	
Thyroid diseases, except Levothyroxine treatment of hypothyroidism if not 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 had the potential of affecting body weight incl. food supplements	Use currently or within the previous 3 months of prescription or over the counter medication that had the potential of affecting body weight incl. food supplements
Exceptions related to medical conditions: I) Cholesterol or blood pressure lowering medication were allowed if the participant's dose had not changed during the last 3 months II) low dose antidepressants if they, in the judgement of the investigator, did not affect weight or study participation. III) Levothyroxine for treatment of hypothyroidism if on a stable dose for at least 3 months	
Laboratory screening[1]	
Glucose >7.0 mmol/L	-
Haemoglobin: women; <7.5 mmol/L (Copenhagen, Maastricht, Navarra, Athens) men; <8.5 mmol/L (Copenhagen, Maastricht) and <8.1 mmol/L (Navarra, Athens)	
For Maastricht participants only: Creatinine <50 µmol/L and >100 µmol/L ALT >34 IU	

[1]Fasting blood sample was collected from adults and locally analysed to assess glucose and haemoglobin levels, and some additional values at Maastricht.
ALT, Alanine transaminase; CVD, cardiovascular diseases; IU, international unit

Randomisation

After screening, eligible participants were randomly assigned to one of the two intervention groups in a 1:1 ratio by a site-specific randomisation list created by a person in Copenhagen not involved in the trial. The randomisation was stratified by gender, age (<40 years or ≥40 years) and BMI (<30 or ≥30 kg/m^2), and stratification was implemented by sequentially assigning families and adults from each stratum to the two interventions in blocks of 4, using the software R. Each household was randomised to the same intervention determined by the oldest member of the household. Although randomisation was done after screening, it is not revealed to the household/participant before completion of the initial 2-month period.

Intervention

This 1-year trial is divided into two periods of 2- and 10-months duration (Figure 1) with the second period being the randomised intervention period.

Two-month period

In the initial 2-month period, adults - regardless of randomisation - receive a low-energy diet (LED) (Cambridge Weight Plan, Northants, United Kingdom). If the clinically relevant criteria for WL of ≥5% [21] is not achieved, the participant will be excluded. During the 2-month period, adults visit the intervention site 2-3 times for collection of LED products, weighing and dietetic counselling. The LED consists of 3,347-4,184 kJ/d, 15-20 E% fat, 35-40 E% protein and 45-50 E% carbohydrate. Four products per day will be provided as shakes, soups, ready-to-drink products and bars. Additionally, 200 g tomatoes, 125 g cucumber, 50 g lettuce and chewing of maximum 6 pieces of sugar-free chewing gum or pastilles per day are allowed. For some adults (e.g. BMI >40 kg/m^2 or achieving a BMI ≤23 kg/m^2 during the LED without a wish to lose more weight), the LED may be supplemented with milk/yoghurt, but only if it is expected that the required 5% WL can be achieved.

In the initial 2-month period, children are encouraged to follow the dietary recommendations of the American Academy of Paediatrics on the prevention, assessment and treatment of overweight and obesity.[22] The goal is to obtain weight stability, which will reduce BMI-for-age z-score. For children, no weight criterion exists. Therefore, all children can continue into the WM period as long as their adult family member is included. Children are welcome to visit the intervention site for weighing and dietician counselling, however it is not mandatory.

Ten-month period with S&SEs and sugar diets

During the 10-month randomised intervention period, dietary counselling sessions will be practiced as individual (i.e. household) counselling sessions at months 2 and 6 and when Covid-19 restrictions allow in intervention groups (months 4 and 9). Otherwise, individual counselling sessions will be scheduled. The goals are to maintain WL for adults and BMI-for-age z-score for

children. Further reduction in weight or BMI-for-age z-score is allowed, if the participant is compliant with the intervention, but counselling sessions will only cover maintenance aspects.

The two intervention diets are I) a healthy diet with <10 E% sugar allowing foods and drinks with S&SEs (S&SEs group) and II) a healthy diet with <10 E% sugar not allowing foods and drinks with S&SEs (Sugar group). Both diets are *ad libitum* and S&SEs cover all types (artificial, natural, low-calorie, sugar alcohols, non-caloric) available on the market. To secure dietary adherence calculation of maximum sugar intake (g) will be based on a diet with 9.5 E% sugar. The maximum allowed sugar intake will be calculated individually based on body weight at month 2 (re-calculated at month 6), using the formula by Henry [23] multiplied by the physical activity level (PAL). A unit system for the sugar and S&SEs intake has been developed, where individual maximum sugar intake is converted to a certain number of units per day (and week) (1 unit corresponding to 10 g sugar). One unit of S&SE product in the S&SE group is equal to the amount - in weight or volume - of one unit sugar-rich product in the Sugar group. For the S&SE group, as many sugar-containing products as possible should be replaced by S&SE products. Food exchange lists, covering categories listed in Table 2 including pictures of products, amounts and units per product, guide the participants in the two groups. Additional details and examples of the two interventions are provided in Table 3. Due to the characteristics of the study, blinding is not possible, however all effort to blind study staff taking measurements and persons doing statistical analysis will be done.

Table 2: Foods and drinks relevant for the 10-month randomized intervention period

Category	Examples
Drinks	Carbonated soft drinks, fruit juice, non-carbonated soft drinks, cocoa powder, mixture of fruit syrup and water, energy drinks, pre-packed juices and nectars, protein shakes, energy drinks
Milk products	Flavoured yoghurts, yoghurt drinks, milk shakes, chocolate milk, fermented milk, cold butter milk
Breakfast cereals	Breakfast cereals, muesli, cereals bars, rolled oats
Sugar, honey and marmalade	Sugar, syrup, honey, marmalade, jam, compote
Chocolate and bars	Chocolate with and without filling, chocolate bars, chocolate/hazelnut paste/spread, thin sliced chocolate
Desserts	Pudding, mousse, cold soufflé, custard, strained stewed fruit, Greek jelly, pancakes
Ice cream	Ice cream, sorbet, ice lolly
Candy	Wine gum, liquorice, Bon-bon mix, marshmallow, marzipan
Cake and biscuits	Cake, cookies, biscuits, Danish pastry, sponge cake

Table 3: Description of diets in the 10-month randomized intervention period

	Sugar group	S&SE group
Sugar-containing products	<10 E% added sugar.	<10 E% added sugar and as little as possible.
S&SE products	Not allowed. Except for up to 2 pieces of sugar-free chewing gum per day.	Allowed. And without any restrictions on specific types of S&SEs.
Units	Consumption of a maximum number of units (corresponding to 9.5 E% added sugar) of sugar-containing products each day/week.	Unit calculation (corresponding to 9.5 E% added sugar in weight/volume) will guide the participant to ensure intake of <10 E% added sugar. As many sugar-containing products in the diet as possible should be replaced with S&SE-containing products. Ideally, the amount of S&SEs products corresponding to the maximal units from sugar-containing products should be consumed. However, if a participant experiences an adverse event, they are recommended to consume less than the calculated units and change to other S&SE products (e.g. avoid sugar-alcohols).
Example	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. 5 units per day or 35 units per week is then the maximum allowed intake of added sugar for this participant.	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. Ideally, this participant should consume 5 units per day or 35 units per week of S&SE containing products. One unit is equivalent to 1 unit in the sugar group (in weight or volume).

E%, energy percentage; S&SEs, sweeteners and sweetness enhancers.

Compliance:

Participants are required to record intake of all foods and drinks (pen and paper) for 4 days (3 weekdays and 1 weekend day) at months 0 and 12 with information on time, type/brand names, cooking and processing methods, weight or household measures. Daily average intake of energy, macro- and micro-nutrients will be calculated by national nutritional software in the 4 intervention sites. From the food records, amount and intake of sugar and S&SEs in units from the products listed in Table 2 is also assessed at months 0 and 12. Furthermore, adults complete a food frequency questionnaire about intake of sweet products (sFFQ) during the past month and they collect 24-hour urine samples at months 0, 6 and 12. Urinary biomarkers of S&SEs (acesulfame-K, saccharin, sucralose, cyclamate, and steviol glycoronide) as well as fructose and sucrose will be analysed by Wageningen University, Netherlands, and urinary excretion of urea/nitrogen will be analysed locally.

Data collection and outcomes

Including information meeting, screening, counselling sessions and CIDs, the trial consists of a minimum of 10 and 6 visits for adults and children, respectively. Data is collected according to standard operation procedures (SOPs) and Table 4 shows activities/data collection at each visit. Most data will be collected at months 0, 2, 6 and 12 where participants have fasted for a minimum of 10 hours, and avoided intensive physical exercise, coffee and smoking for 12 hours prior to the CIDs.

Table 4: Flow chart for adults (A) and children (C) (full sampling at months 0 and 12)

	Pre-screening	Information meeting	Screening	Baseline		*2-month period (CID1-CID2)*		*10-month randomised intervention period (CID2-CID4)*			1-year assessment
CID	-	-	-	**CID1**	-	-	**CID2**	-	**CID3**	-	**CID4**
Visit	-	**V0**	**V1**	**V2**	**V3**	**V4**	**V5**	**V6**	**V7**	**V8**	**V9**
Month	-		-	**0**	**0.5**	**1**	**2**	**4**	**6**	**9**	**12**
Inclusion/exclusion criteria	A+C		A+C								
Signing Informed Consent		A+C[0]									
Med. hist., medication etc.			A+C								
Randomisation of the oldest family/household member			A								
Supervision/counselling				**A+C[1]**		A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**
Collection of LED products				**A[#]**	A[#]	A[#]	**A[#]**				
Body weight and height[4]			A+C	**A+C**	A[3]	A[3]	**A+C**	A[3]	**A+C**	A[3]	**A+C**
Waist and hip circumference				**A+C**			**A+C**		**A+C**		**A+C**
Body composition				**A+C[5]**			**A**				**A+C[5]**
Blood pressure and heart rate			A+C	**A+C**			**A+C**		**A+C**		**A+C**
Fasting blood samples			A[6]	**A+C**			**A+C[7]**		**A+C**		**A+C**
Adverse events and concomitant medication				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity (skin prick test)				**A**							**A**
24h urine collection (content of S&SEs)				**A**					**A**		**A**
Faecal spot sample				**A**			**A**		**A**		**A**
4-day dietary record				**A+C**							**A+C**
Questionnaires (electronic platforms):											
General background questionnaire				**A+C**							
Physical activity				**A+C**							**A+C**
Three factor eating questionnaire				**A+C**							**A+C**
Leeds food preference questionnaire				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity				**A+C**							**A+C**
Craving for sweet taste				**A**							**A**
Perception of S&SEs				**A**					**A[8]**		**A[8]**
Control of eating				**A**			**A**		**A**		**A**
Subjective appetite sensations				**A**			**A**		**A**		**A**
Sweet food frequency questionnaire (FFQ)				**A**							**A**
Diet satisfaction				**A**					**A**		**A**
Perception and evaluation of the intervention									**A**		**A**
Quality of life				**A**							**A**
Puberty				**C**					**C**		**C**

[#]Adults will collect LED products from the intervention site every 2nd or 3rd week during the 2-month period. At months 0.5 and 1.5 (optional) the adults will be weighed and have the opportunity to consult a dietician.
[0]For children, the informed consent is signed by the parents/guardians.
[1]Individual/family counselling is preferably scheduled at the same day as the CID.
[2]Group counselling, children participation is preferred, but not mandatory.
[3]Fasting is not required for this body weight measurement.
[4]Height is only measured at screening for adults.
[5]At University of Maastricht, body composition is not measured in children.
[6]At screening, fasting blood samples will be analysed at each intervention site. All other blood samples are analysed at the Central Laboratory (Bioiatriki).
[7]At University of Maastricht, a fasting blood sample is not drawn from children at CID2.
[8]A shorter version of the questionnaire is used at CID3-4.
A, adult; C, child; CID, clinical investigation day; LED, low-energy diet; S&SEs, sweeteners and sweetness enhancers.

Primary outcomes

This trial has two independent primary outcomes. The primary outcome for efficacy is 1-year change in body weight. The primary outcome for safety is 1-year change in gut microbiota composition associated with impaired health (e.g. change in microbial beta-diversity and composition). Both outcomes relate only to adults, and hence the required sample size was calculated for adults only.

Body weight

Body weight is measured to the nearest 0.1 kg using a digital scale with the participant wearing underwear/light clothes. Fasting body weight will be measured at screening and CIDs, however fasting is not required, when body weight is measured at other visits.

Gut microbiota

Gut microbiota composition will be assessed from faecal spot samples collected at home prior to all CIDs. Samples are immediately frozen (-20 °C) and later transported to the intervention site in cooling bags, whereafter they are stored at -80°C. Samples will be analysed targeting the V3-V4 regions of 16s rRNA genes by Illumina sequencing at Maastricht University, Netherlands.

Secondary outcomes

Secondary outcomes include changes in anthropometry and body composition (for children, BMI-for-age z-score), risk factors for T2D and CVD, allergenicity, adverse events (AE), and concomitant medication. Additionally, some secondary outcomes will be assessed in adult sub-groups e.g. gut-brain signalling markers, postprandial energy expenditure and substrate oxidation, physical activity, liver fat, adipose tissue and lipid metabolism, brain reward, insulin sensitivity markers, and composition and functionality of the human gut microbiota in vitro. Furthermore, children's gut microbiota composition may be analysed depending on the final sample size (Table 5).

Anthropometry

Height is measured to the nearest 0.5 cm using a stadiometer at screening and for children at all CIDs. For adults, BMI is calculated as body weight (kg) / height^2 (m^2). For children, the WHO AnthroPlus software (www.who.int/tools/growth-reference-data-for-5to19-years/application-tools) is applied to calculate BMI-for-age percentile and z-score. Waist and hip circumferences are measured twice with a non-elastic tape measure on the skin to the nearest 0.5 cm, and the average is calculated. Waist circumference is measured halfway between the lowest rib and iliac crest during exhalation. Hip circumference is measured as the largest circumference in the area around the buttock. Dual-energy X-ray absorptiometry (DXA) scans are performed in underwear to assess body composition including fat percentage, fat mass and fat free mass.

Blood pressure and heart rate

After minimum 5 minutes rest in a sitting position, blood pressure (mmHg) and heart rate (beats per minute) are measured three times on the right arm with an automatically inflated cuff. An average is calculated from the last two measurements when the two measurements differ with ≤5 mmHg. If either the systolic or diastolic blood pressure differ by >5 mmHg, a fourth measurement is performed and the average calculated from the third and fourth measurement.

Blood samples

Fasting venous blood samples are drawn at all CIDs, except at month 2 for children at Maastricht due to Ethical concerns. Serum samples are collected for analyses of lipids (triglycerides, total, low-density lipoprotein and high-density lipoprotein cholesterol), alanine aminotransferase, aspartate aminotransferase, insulin, C-reactive protein and immunoglobulin E. Plasma is collected for glucose analysis and full blood for HbA1c analysis. All samples are stored locally at -80 ºC until shipment to the central lab at Bioiatriki S.A., Greece.

Skin prick test

For adults, a skin prick test is performed on the forearm. One drop of the allergens hazel, alder, birch, grass mix, artemisia absinthium, ragweed, alternaria, moulds mix, cat, dog,

dermatophagoides pteronyssinus and dermatophagoides mix as well as positive and negative control solutions are applied. The response is recorded after15 minutes.

Adverse events and concomitant medication

All AEs experienced after inclusion and during the trial are registered. At CIDs, the participant is asked if he/she has noticed any unfavourable events since the last CID. During the 10-month randomised intervention period, participants - regardless of intervention - are asked directly about certain AEs that may be related to consumption of S&SE i.e. gastrointestinal symptoms and headache. All medication necessary for the participants' health and which is not in the protocol exclusion criteria may be continued during the trial. At CIDs, the participant is asked if he/she has taken any new medicine or has changed dosage of already registered medicine.

Other outcomes

Questionnaires are used to obtain information about sociodemographic characteristics such as education, occupation, household income etc., physical activity, quality of life, and to investigate subjective neuro-behavioural indices e.g. food preferences and preference for sweet taste, perception of S&SEs, cravings, subjective appetite sensations, and perception and evaluation of the 10-month randomised intervention period. Furthermore, puberty is assessed for children. All questionnaires are prepared in English and later translated into local language. The majority of questionnaires will be delivered by a Questionnaire Delivery Platform (QDP) implemented by NetUnion, Switzerland. At baseline all questionnaires are completed at the intervention sites, but before other CIDs adults can complete those delivered by the QDP at home prior to the CID. Children always complete all questionnaires at the intervention site. Two questionnaires are always completed at the intervention site; one about perception of S&SEs (delivered by the Qualtrics platform via a weblink) and one about food rewards assessed by the Leeds Food Preference Questionnaire (E-prime software).

Sub-groups and sub-studies

Some of the secondary and other outcomes are collected in sub-studies and in sub-groups of different participants. Table 5 presents outcomes only investigated in sub-groups.

Outcome	Measurements and method	Participants	Time points of data collection			
			Baseline	After WL		After WM
			Month 0 CID1	Month 2 CID2	Month 6 CID3	Month 12 CID4
Sub-studies (include an intervention)						
Brain reward activity	Brain activity is measured by fMRI after consumption of a drink with sugar, S&SEs, water	Sub-study incl. a sub-group of adults at Maastricht	A	A		A
Postprandial responses (energy expenditure, substrate oxidation, blood biochemistry and appetite)	Indirect calorimetry, blood sampling, appetite sensation based on VAS and *ad libitum* energy intake after consumption of a drink with S&SE or water	Sub-study incl. a sub-group of adults at Copenhagen	A	A	A	
Sub-groups						
Physical activity	7-day measurement by accelerometer	Adults at Maastricht	A		A	A
Gut-brain signalling markers	Analyses of GLP-1, CCK and ghrelin from fasting blood samples	Adults at Copenhagen and Maastricht HUA?	A	A	A	A
Liver fat	^{1}H-MRS	Sub-group of adults at Maastricht	A	A		A
Adipose tissue function and lipid metabolism	Adipocyte morphology, ex vivo lipolysis, gene and protein expression analyses of adipose tissue samples (biopsy)	Adults at Maastricht	A	A		A
Insulin sensitivity markers	Indices e.g. HOMA-IR, Matsuda index, Disposition index etc. calculated from a 7-point OGTT	Adults at Maastricht				
Gut microbiota	16S rRNA illumine sequencing of faecal samples	Children at Maastricht	C		C	C
Composition and functionality of the human gut microbiota in vitro	Microbial metabolites e.g. SCFA and 16S rRNA illumine sequencing of faecal samples	Sub-group of adults at Maastricht	A			

A, adults; C, children; CCK, cholecystokinin; CID, clinical investigation day; fMRI, functional Magnetic Resonance Imaging; GLP-1, glucagon-like peptide 1; ^{1}H-MRS, Proton Magnetic Resonance Spectroscopy; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance, OGTT, oral glucose tolerance test; SCFA, short chain fatty acids; S&SEs, sweeteners and sweetness enhancers; VAS, visual analogue scales; WL, weight loss, WM, weight maintenance.

Statistical methods

Sample size determination

The sample size calculation is based on adults for the two primary outcomes. For body weight, a clinically meaningful effect of 1.5 kg placebo subtracted body weight has previously been approved by the European Food Safety Authority.[24] Based on a similar trial [25] we estimated that a difference of 1.5 kg with a SD of ± 3.5 kg, a 90% power, a two-sided α of 0.05, would require 231 completers. With an estimated drop-out of 30% a minimum of 330 adult participants

should be included. For change in gut microbiota, a ± 10% change in 20 of the 50 most abundant operational taxonomic units (OTUs) with an alpha of <0.05%, would require 100 complete samples. The inclusion of at least 330 adults (approximately 25% of the participants per intervention site) is therefore also sufficient to detect possible changes in gut microbiota.

Statistical analysis plan

As part of the SWEET project a statistical analysis plan has been developed. For body weight, 1-year change between the two interventions will be analysed by analysis of covariance (ANCOVA) linear mixed model, with change in body weight as response; treatment group and relevant covariates e.g. age, gender and BMI, are fixed effects, and participant ID and intervention site are random (intercept) effects. Intention-to-treat principle will be applied on those completing the initial 2-month period. Additionally, complete-case analyses (all dropouts omitted) and per protocol analyses (only compliant participants) as well as analyses including additional covariate adjustments and intermediate time points will be applied.

For gut microbiota, 1-year change in microbial diversity and microbial composition (relative abundance at phyla and genera level) will be analysed. Paired Wilcoxon test is used to study within intervention changes in relative abundance, and linear mixed models with Benjamin-Hochberg correction for multiple testing will be used for between intervention comparisons.

Data will be presented with the use of standard descriptive statistics shown as mean (SD) or median (Q1:Q3) for normally and non-normally distributed data, respectively, and categorical data by percentages. Results will be presented as mean difference in changes ± SEM or 95% CIs and p-values when relevant. A statistical level of 0.05 will be applied and graphical models will be carried out to assess model assumptions. When relevant, transformation e.g. logarithm will be applied or non-parametric statistical tests will be performed.

For secondary outcomes on continuous data, the main analysis will compare the 1-year mean change between the two treatment groups by use of the ANCOVA-type linear mixed model defined above without any multiplicity adjustment or imputation of missing values (i.e. available-

case analyses). Additional sensitivity analyses may be carried out as appropriate in the same way as for the primary outcome. Furthermore, analysis of repeated measures will be performed using linear mixed models including time×treatment interaction, time, and treatment effects, covariates (e.g. age, gender, BMI) as fixed effects, and participant ID and intervention sites as random effect. In case of significant time×treatment interaction, differences between treatments will be identified per time point. Mean changes will be compared between the groups using the estimated mean difference and approximate t-tests derived from the fitted linear mixed models (assuming a two-sided alternative). For secondary categorical outcome e.g. (yes/no, 0/1/2, etc.) logistic or ordinal mixed effects model including the same fixed and random effects as the linear mixed models will be used.

ETHICS AND DISSEMINATION

The trial will be conducted in accordance with the Declaration of Helsinki[26] and this master protocol (version 3.0, 28-Oct-2020) is approved by the responsible national/regional committee in the 4 countries from where consent to all previously and future amendments to the protocol was and will be obtained. All adults receive the LED products free of charge. At Copenhagen, Navarra and Athens participants will not receive reimbursement for their participation. At Maastricht, travel expenses and financial compensation are provided to all eligible participants (125 Euros for adults without child(ren) and 250 Euros for one adult and one child + 80 Euros per extra family member).

There are no risks related to the dietary interventions, however discomfort may occur. The LED (not provided for children) contains all needed nutrients, but only little energy and therefore adults may experience headaches, dizziness, tiredness and nausea particularly in the first few days. Constipation, stomach cramps or more profound nausea can occur and information on this is given before inclusion, however allergic reactions to the LED, are rare. The sugar and S&SEs intervention products are commercially available foods and drinks purchased in the supermarket and no adverse side effects are expected. However, changes in gastrointestinal symptoms e.g.

bloating and excess gas production may occur depending on the participant's habitual intakes of fibre and types of S&SEs e.g. sugar alcohols. At each intervention site a physician can be consulted in case of medical uncertainties.

Some study procedures involve risks, however, the procedures implemented are designed to minimize these. Drawing blood samples will seldom cause harm besides that associated with the insertion, however children will be offered local anaesthetic Emla patches to reduce pain. A maximum of 80 ml and 125 ml of blood is drawn during the 1-year trial for children and adults, respectively. For children, this is less than 1 ml blood/kg body weight per donation which is considered safe. Fertile women will be tested for pregnancy before DXA scanning and excluded from the trial if pregnant. The DXA scans will induce minor radiation (<0.010 mSv per scan). Scanning will be done 2 and 3 times during the 1-year trial for children and adults, respectively, and only one re-scan will be allowed per CID. The skin prick test, only performed in adults, is anticipated to cause very little discomfort. A positive reaction, may cause itching, which will be treated with a salve. In very rare cases a systemic anaphylactic reaction can occur and emergency equipment is in place.

For children, special attention is given to ensure that the child is not forced to participate by the adult family member. Furthermore, a child cannot remain included if the adult family member drops out or is excluded from the trial.

All participants will be insured against injury caused by their participation according to local legal requirements. The trial is monitored by European clinical research infrastructure network (ECRIN) to ensure compliance with the protocol and SOPs. All trial-related information will be recorded, handled and stored safely allowing accurate reporting, interpretation and verification. All data will be collected in a central DataHub at Copenhagen from where pseudo-anonymised data can be requested before 2032 via a data sharing contract. From 2032 fully anonymised data can be transferred. Source data is collected on paper first or is entered directly into the electronic systems e.g. the QDP, the Qualtrics platform and/or the Research Electronic Data Capture

(REDCap) tool hosted at University of Copenhagen.[27,28] REDCap is a secure, web-based software platform designed to support data capture. Source data from DXA scans and analysis of biological material are registered on the device or related hardware, whereas the source data from dietary records (handwritten on paper) will be entered into a national software program for analysis. The sponsor/investigator will provide direct access to source data/documents for inspection.

Results will be published in international peer-reviewed scientific journals regardless of whether the findings are positive, negative or inconclusive.

AUTHORS' CONTRIBUTIONS

The SWEET EU-project was initiated by JCGH, AR, and JH. The protocol for the SWEET intervention trial was written by LK, AR and YM, with contribution from EEB and JAM. AR, YM, EEB, and JAM are principal investigators (PI) at the 4 intervention sites, where LK, SSHA, SN-C, KR and TCA are investigators. EJMF, GF, CEH, TL and HM are responsible for specific methods, platforms or analyses, and MdA is responsible for monitoring of the trial. LK and AR drafted the manuscript and YM, EEB and JAM critically reviewed the manuscript. All authors read and approved the final manuscript.

FUNDING STATEMENT

PI, AR, is also the sponsor (e-mail: ara@nexs.ku.dk, Phone: +45 21 30 69 12, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark). The trial is funded by the Horizon2020 program: *Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability* (acronym: SWEET, grant # 774293) and funding covers salary for project personal, supplies, remuneration, and dissemination of results. The amount is deposited in a project account subject to audits/public revision.

COMPETING INTERESTS STATEMENT

AR has received honoraria from Unilever and the International Sweeteners Association. CEH's research centre provides consultancy to, and has received travel funds to present research results from organisations supported by food and drink companies. JCGH and JH have received project funds from the American Beverage Association. TL works for a company, NetUnion sarl, which has no conflict of interest in the study outcome.

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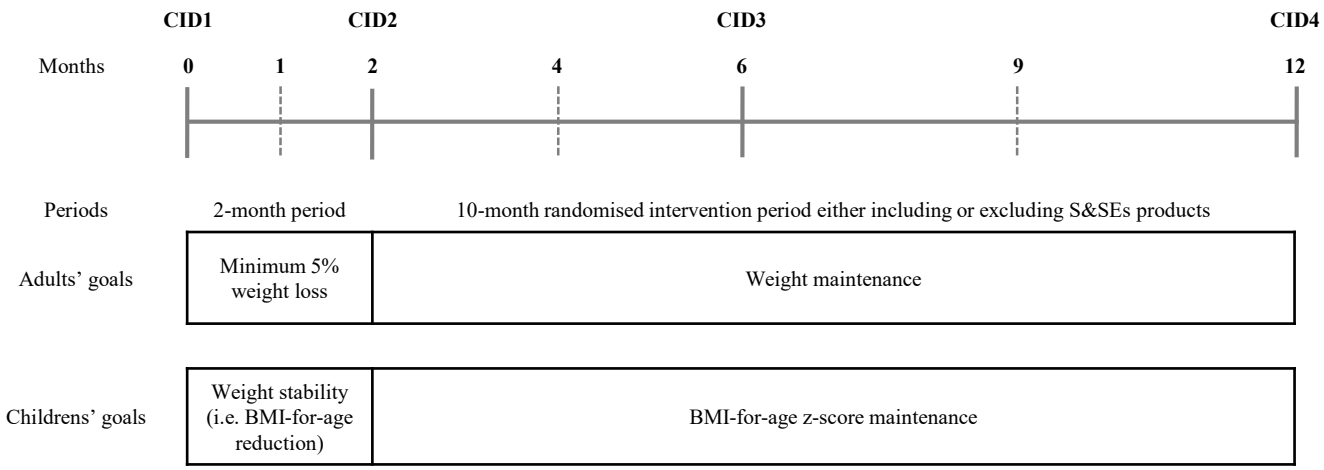
FIGURE LEGEND

Figure 1: Overall study design.
Solid lines are CIDs and dashed lines are dietary counselling sessions where non-fasting body weight of adults is measured. Additionally, LED products for adults will be collected from the intervention site every 2nd or 3rd week during the initial 2-month period.
BMI: body mass index. CID: clinical investigation day; S&SEs, sweeteners and sweetness enhancers.


CID1
CID2
CID3
CID4
Months
0
1
2
4
6
9
12
Periods
2-month period
10-month randomised intervention period either including or excluding S&SEs products
Adults' goals
Minimum 5% weight loss
Weight maintenance
Childrens' goals
Weight stability (i.e. BMI-for-age reduction)
BMI-for-age z-score maintenance

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2+21
	5b	Name and contact information for the trial sponsor	21
	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	21
	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)	21
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	5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6

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, noninferiority, exploratory)	6-7+9
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	6
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)	7-8+ Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11 + Table 2+3
	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)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
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	14-17
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)	Figure 1 +Table 4
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	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
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	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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	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	17
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	20
Statistical methods	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	17-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17-18
	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)	18

Methods: Monitoring			
Data monitoring	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	20
	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	NR
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	16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NR
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)	18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NR
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	19-20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
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	18-19

Dissemination policy	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	4+20
	31b	Authorship eligibility guidelines and any intended use of professional writers	NR
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	The master version can be delivered by request
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	NR

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Protocol for a multicentre, parallel, randomised, controlled, trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in overweight adults and children. The SWEET project.

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Manuscript ID	bmjopen-2022-061075.R2
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Primary Subject Heading:	Nutrition and metabolism
Secondary Subject Heading:	Evidence based practice, Diabetes and endocrinology, Public health

Keywords:	Nutrition < TROPICAL MEDICINE, DIABETES & ENDOCRINOLOGY, Microbiology < NATURAL SCIENCE DISCIPLINES, MICROBIOLOGY, Allergy < THORACIC MEDICINE

Protocol for a multicentre, parallel, randomised, controlled, trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in overweight adults and children. The SWEET project.

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Word count: 4572

Keywords: sugar, body weight, gut microbiota, weight loss, weight maintenance, weight loss maintenance, obesity, anthropometry, type 2 diabetes, cardiovascular diseases, allergenicity.

ABSTRACT

Introduction

The aim of this randomised controlled trial (RCT) is to investigate if prolonged consumption of sweeteners and sweetness enhancers (S&SEs) within a healthy diet will improve weight loss maintenance and obesity related risk factors, and affect safety markers compared to sugar.

Methods and analysis

SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is a 1-year multicentre RCT including at least 330 adults with overweight (18-65 years, body mass index (BMI)>25 kg/m^2) and 40 children (6-12 years, BMI-for-age>85th percentile). In an initial 2-month period, adults will consume a low-energy diet with the aim to achieve ≥5% weight loss. Children are advised to consume a generally healthy diet to maintain body weight, thus reducing their BMI-for-age z-score. In the following 10 months, participants will be randomised to follow a healthy *ad libitum* diet with or without S&SE products. Clinical investigations are scheduled at baseline, after 2, 6, and 12 months. The primary outcomes are body weight for efficacy and gut microbiota composition (in relation to metabolic health) for safety, both in adults. Secondary outcomes include anthropometry, risk markers for type-2-diabetes and cardiovascular diseases, questionnaires including e.g. food preferences, craving and appetite, and tests for allergenicity.

Ethics and dissemination

The trial protocol has been approved by the following national ethical committees; The research ethics committees of the capital region (Denmark), approval code: H-19040679, The medical ethics committee of the University Hospital Maastricht and Maastricht University (the Netherlands), approval code: NL70977.068.19/METC19-056s, Research Ethics Committee of the University of Navarra (Spain), approval code: 2019.146 mod1, Research Ethics Committee of Harokopio University (Greece), approval code: 1810/18-06-2019. The trial will be conducted in accordance with the Declaration of Helsinki. Results will be published in international peer-

reviewed scientific journals regardless of whether the findings are positive, negative, or inconclusive.

Trial registration number: NCT04226911.

Strengths and limitations of this trial

- The trial investigates long-term effects of S&SEs compared to sugar in the contexts of an *ad libitum* healthy diet including both foods and drinks.
- Weight maintenance after weight loss is difficult to achieve and in this trial the effects of consuming S&SE foods and drinks compared to sugar during 10-month weight maintenance after 2-month weight loss are investigated.
- A broad range of measurements related to health and safety, appetite and food preferences are included to address concerns raised in relation to consumption of S&SEs.
- This is a multicentre trial covering Northern, Central and Southern Europe, thereby reflecting different geographic distributions of adult and childhood obesity in Europe.
- A potential limitation is that the number of included children was reduced through the recruitment period, but this will not affect the 2 primary outcomes as sample size determination was done exclusively for adults.

INTRODUCTION

Obesity is a major global health problem giving rise to increased risk of non-communicable diseases such as type-2-diabetes (T2D) and cardiovascular diseases (CVD).[1] Sustaining energy balance is critical to maintain body weight. However, sugar contributes to the energy density of most diets and may therefore promote a positive energy balance.[2] In 2015, the World Health Organization (WHO) strongly recommended that free sugar intake should be <10 energy percentage (E%) and preferably <5 E% as a conditional recommendation.[2] The latter is still not fulfilled by large parts of the population, including Denmark,[3,4] Greece,[5] Spain,[6] and the Netherlands.[7]

One often-recommended approach to reduce sugar intake is to replace sugar with sweeteners or sweetness enhancers (S&SEs). The use of S&SEs allows products to retain their palatability without the associated calories, creating a perception of a 'healthier' product.[8] Although drinks often constitute the largest part of S&SEs products consumed, S&SEs products also include foods. In the US and worldwide, the consumption of S&SEs products such as desserts, gums and breakfast foods has increased.[9] However, foods with S&SEs have been less extensively investigated. S&SEs comprise a variety of compounds with proposed negative effects on health parameters,[10] however evidence is conflicting. For example, S&SEs have been claimed to result in detrimental effects on appetite, body weight, glucose metabolism, and gut microbiota.[8] In contrast, several systematic reviews and meta-analysis found no detrimental effects on appetite and body weight – rather on the contrary[11–13] and interestingly, a large 1-year study found S&SEs to be superior to water for weight loss and weight maintenance.[14] Not all reviews have come to the same conclusion, but selective citation of the different studies could be the cause.[15] In relation to postprandial glycemia and insulinemia no differences were observed between S&SEs and controls in recent systematic reviews and meta-analysis.[16,17] Following consumption of S&SEs changes in the gut microbiota composition and functionality have been debated as a food safety issue, because some changes in specific bacteria have been associated with diseases and risk markers of diseases.[18,19] As an example, a change in microbial

composition after 7 days consumption of saccharin has been associated with impaired glucose homeostasis.[20] However, that study included only 7 human participants, there was no control group, and conclusions were based on a post-hoc division of responders and non-responders.[20] In general, safety concerns have derived from animal studies using S&SE doses far above habitual intake in humans. Long-term controlled human intervention studies are therefore warranted.[19]

Aim and objectives

The overall aim of the randomised controlled trial (RCT) SWEET (Sweeteners and sweetness enhancers: Prolonged effects on health, obesity and safety) is to investigate the efficacy and safety of combined (foods and drinks) and prolonged use of S&SEs - as part of a whole healthy *ad libitum* diet approach - in a population of overweight adults and children. The 2 primary outcomes on efficacy and safety will be assessed in adults by 1-year changes in body weight and 1-year changes in gut microbiota related to metabolic health outcomes, respectively. Secondary objectives concern effects on obesity-related risk factors such as fat mass, glucose metabolism, and lipidemia, as well as safety aspects such as allergenicity. Other outcomes include appetite sensations, food cravings, food preferences, and preference for sweet taste.

Hypothesis

We hypothesize that prolonged use of S&SEs in foods and drinks will increase palatability of the diet and thereby increase compliance to the recommendations for a healthy sugar-reduced diet resulting in improved body weight control. Further, we hypothesize that there will be no safety concerns using S&SEs in the long-term.

METHODS AND ANALYSIS

Study design

SWEET is conducted in 4 intervention sites; Athens, (Harokopio University of Athens, Greece), Copenhagen, (University of Copenhagen, Denmark), Maastricht, (Maastricht University, the

Netherlands), and Navarra (University of Navarra, Spain) covering North, Central, South and East Europe, thereby reflecting different geographic distributions of obesity in Europe. In the 1-year RCT both adults and families (at least 1 adult and 1 child) are included. The RCT consists of an initial 2-month period followed by a 10-month randomised 2-armed parallel intervention period. For adults, the goals in these periods are first to achieve a weight loss (WL) and second to maintain the WL. For children, the goals are first to achieve weight stability and second to maintain BMI-for-age z-score. The 10-month randomised intervention period will be carried out by using a "fading visit" approach (Figure 1). During the RCT, all participants will undergo 4 clinical investigation days (CIDs) and will be supervised by dieticians individually/familywise and/or in groups at least every 3rd month.

Originally, a 1-year follow up period was planned after the 10-month intervention period; however, it was omitted due to recruitment delay caused by the Covid-19 pandemic. Furthermore, the initial plan was to include at least 1 child per adult (i.e. only families). However, recruitment turned out to be very difficult and the strategy was changed to also include adults without children, because the primary outcomes and sample size determinations were based solely on adults. Screening visits were conducted between 29-Jun-2020 and 27-Sep-2021, and the last patient last visit (CID at month 12) is scheduled for 30-Sep-2022.

Patient and public involvement

Neither patients nor the public were involved in the design and conduct of the study and they will not be involved in interpretation, reporting, or dissemination of the trial.

Participants

Recruitment and screening

Participants were recruited continuously by multiple routes e.g. web-pages, social media, newspapers, and registries (local databases or civil registration numbers). Potential adult participants were pre-screened by phone and answered questions on behalf of their child(ren). If still eligible and interested after pre-screening, they received written information and were

invited to an information meeting. After the information meeting, an informed consent form and a general data protection regulation form were signed by the adult participant, and for children by the parents, or person(s) having custody and the site-PI or delegated staff. Thereafter, the screening visit was scheduled. Participants were screened in the fasting state, where all in- and exclusion criteria were assessed. The recruitment has ended with inclusion of 341 adults and 38 children.

Eligibility criteria

Adults (men and women), 18-65 years, BMI≥25 kg/m^2 and children (boys and girls), 6-12 years, and BMI-for-age>85th percentile were included. Children were only included if they had an eligible adult family member (i.e. as a family) - a biological relationship was not required. However, it was required that the family lived in the same household at least 4 days/week. Participants were required to have a regular consumption of sugar-containing/sugar-sweetened products and be motivated and willing to be randomised to any of the 2 intervention groups. All exclusion criteria are listed in Table 1. In- and exclusion criteria were assessed at screening, however, the site-PI or delegated personnel has the right to terminate participation at any time if deemed in the participant's best interest, and children are excluded if their adult family member's participation is discontinued.

Table 1: List of exclusion criteria

Adults	Children
General	
Weight change >5% 2 months prior to screening	Intensive physical training (>10 hours of per week)
Surgical treatment of obesity	Self-reported eating disorders
Blood donation <3 months prior to study initiation	Intolerance and allergies expected to interfere with the study
Change in smoking habits during the last month. (Smoking was allowed and monitored throughout the study)	Insufficient communication with national language
Regularly drinking >21 (men) or >14 (women) units of alcohol per week	Inability, physically or mental, to comply with the procedures required by the study protocol
Intensive physical training (>10 hours of per week)	Participant's general condition contraindicates continuing the study
Self-reported eating disorders	Simultaneous participation in other clinical intervention studies
Intolerance and allergies expected to interfere with the study	
Self-reported drug abuse within the previous 12 months	
Night- or shift work that ends later than 11 PM	
For women: Pregnancy, lactation	
Persons who do not have access to either (mobile) phone or Internet	
Insufficient communication with national language	
Inability, physically or mental, to comply with the procedures required by the study protocol	
Participant's general condition contraindicates continuing the study	

Adults	Children
Simultaneous participation in other clinical intervention studies	
Medical conditions	
Diagnosed diabetes mellitus	Diagnosed diabetes mellitus
Medical history of CVD (e.g. current angina; myocardial infarction or stroke within the past 6 months; heart failure; symptomatic peripheral vascular disease)	Other diseases that may influence the study outcomes
Systolic blood pressure above 160 mmHg and/or diastolic blood pressure above 100 mmHg (measured at screening) whether on or off treatment for hypertension	
Significant liver diseases e.g. cirrhosis (fatty liver disease allowed)	
Malignancy which was active or in remission for less than 5 years after last treatment (local basal and squamous cell skin cancer allowed)	
Active inflammatory bowel disease, celiac disease, chronic pancreatitis or other disorder potentially causing malabsorption	
Thyroid diseases, except Levothyroxine treatment of hypothyroidism if not 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 had the potential of affecting body weight incl. food supplements	Use currently or within the previous 3 months of prescription or over the counter medication that had the potential of affecting body weight incl. food supplements
Exceptions related to medical conditions: I) Cholesterol or blood pressure lowering medication were allowed if the participant's dose had not changed during the last 3 months II) low dose antidepressants if they, in the judgement of the investigator, did not affect weight or study participation. III) Levothyroxine for treatment of hypothyroidism if on a stable dose for at least 3 months	
Laboratory screening[1]	
Glucose >7.0 mmol/L	-
Haemoglobin: women; <7.5 mmol/L (Copenhagen, Maastricht, Navarra, Athens) men; <8.5 mmol/L (Copenhagen, Maastricht) and <8.1 mmol/L (Navarra, Athens)	
For Maastricht participants only: Creatinine <50 µmol/L and >100 µmol/L ALT >34 IU	

[1]Fasting blood sample was collected from adults and locally analysed to assess glucose and haemoglobin levels, and some additional values at Maastricht.
ALT, Alanine transaminase; CVD, cardiovascular diseases; IU, international unit

Randomisation

After screening, eligible participants were randomly assigned to 1 of the 2 intervention groups in a 1:1 ratio by a site-specific randomisation list created by a person in Copenhagen not involved in the RCT. The randomisation was stratified by gender, age (<40 years or ≥40 years) and BMI (<30 or ≥30 kg/m^2), and stratification was implemented by sequentially assigning families and adults from each stratum to the 2 interventions in blocks of 4, using the software R. Each household was randomised to the same intervention determined by the oldest member of the

household. Although randomisation was done after screening, it was not revealed to the household/participant before completion of the initial 2-month period.

Intervention

The 2 trial periods are illustrated in Figure 1.

Two-month period

In the initial 2-month period, adults - regardless of randomisation - received a low-energy diet (LED) (Cambridge Weight Plan, Northants, United Kingdom). If the clinically relevant criteria for WL of ≥5% [21] was not achieved, the participant was excluded. During the 2-month period, adults visited the intervention site 2-3 times for collection of LED products, weighing and dietetic counselling. The LED consisted of 3,347-4,184 kJ/d, 15-20 E% fat, 35-40 E% protein and 45-50 E% carbohydrate. Four products per day were provided as shakes, soups, ready-to-drink products, and bars. Additionally, 200 g tomatoes, 125 g cucumber, 50 g lettuce, and chewing of maximum 6 pieces of sugar-free chewing gum or pastilles per day were allowed. For some adults (e.g. BMI >40 kg/m^2 or achieving a BMI ≤23 kg/m^2 during the LED without a wish to lose more weight), the LED was supplemented with milk/yoghurt, but only if it was expected that the required 5% WL could be achieved.

In the initial 2-month period, children were encouraged to follow the dietary recommendations of the American Academy of Paediatrics on the prevention, assessment and treatment of overweight and obesity.[22] The goal was to obtain weight stability, which would reduce BMI-for-age z-score. For children, no weight criterion existed. Therefore, all children could continue into the WM period as long as their adult family member was included. Children were welcome to visit the intervention site for weighing and dietician counselling, however, it was not mandatory.

Ten-month period with S&SEs and sugar diets

During the 10-month randomised intervention period, dietary counselling sessions will be practiced as individual (i.e. household) counselling sessions at months 2 and 6 and when Covid-

19 restrictions allow in intervention groups (months 4 and 9). Otherwise, individual counselling sessions will be scheduled. The goals are to maintain WL for adults and BMI-for-age z-score for children. Further reduction in weight or BMI-for-age z-score is allowed, if the participant is compliant with the intervention, but counselling sessions will only cover maintenance aspects.

The 2 intervention diets are I) a healthy diet with <10 E% added sugar allowing foods and drinks with S&SEs (S&SEs group) and II) a healthy diet with <10 E% added sugar not allowing foods and drinks with S&SEs (Sugar group). Both diets are *ad libitum* and S&SEs cover all types (artificial, natural, low-calorie, sugar alcohols, non-caloric) available on the market. To secure dietary adherence calculation of maximum sugar intake (g) will be based on a diet with 9.5 E% sugar. The maximum allowed sugar intake will be calculated individually based on body weight at month 2 (re-calculated at month 6), using the formula by Henry [23] multiplied by the physical activity level (PAL). A unit system for the sugar and S&SEs intake has been developed, where individual maximum sugar intake is converted to a certain number of units per day (and week) (1 unit corresponding to 10 g sugar). One unit of S&SE product in the S&SE group is equal to the amount - in weight or volume - of 1 unit sugar-rich product in the Sugar group. For the S&SE group, as many sugar-containing products as possible should be replaced by S&SE products. Food exchange lists, covering categories listed in Table 2 including pictures of products, amounts and units per product, guide the participants in the 2 groups. Additional details and examples of the 2 interventions are provided in Table 3. Due to the characteristics of the study, blinding is not possible, however all efforts to blind study staff taking measurements and persons doing statistical analysis will be done.

Table 2: Foods and drinks relevant for the 10-month randomized intervention period

Category	Examples
Drinks	Carbonated soft drinks, fruit juice, non-carbonated soft drinks, cocoa powder, mixture of fruit syrup and water, energy drinks, pre-packed juices and nectars, protein shakes, energy drinks
Milk products	Flavoured yoghurts, yoghurt drinks, milk shakes, chocolate milk, fermented milk, cold butter milk
Breakfast cereals	Breakfast cereals, muesli, cereals bars, rolled oats
Sugar, honey and marmalade	Sugar, syrup, honey, marmalade, jam, compote
Chocolate and bars	Chocolate with and without filling, chocolate bars, chocolate/hazelnut paste/spread, thin sliced chocolate

Category	Examples
Desserts	Pudding, mousse, cold soufflé, custard, strained stewed fruit, Greek jelly, pancakes
Ice cream	Ice cream, sorbet, ice lolly
Candy	Wine gum, liquorice, bon-bon mix, marshmallow, marzipan
Cake and biscuits	Cake, cookies, biscuits, Danish pastry, sponge cake

Table 3: Description of diets in the 10-month randomized intervention period

	Sugar group	S&SE group
Sugar-containing products	<10 E% added sugar.	<10 E% added sugar and as little as possible.
S&SE products	Not allowed. Except for up to 2 pieces of sugar-free chewing gum per day.	Allowed. No restrictions on specific types of S&SEs.
Units	Consumption of a maximum number of units (corresponding to 9.5 E% added sugar) of sugar-containing products each day/week.	Unit calculation (corresponding to 9.5 E% added sugar in weight/volume) will guide the participant to ensure intake of <10 E% added sugar. As many sugar-containing products in the diet as possible should be replaced with S&SE-containing products. Ideally, the amount of S&SEs products corresponding to the maximal units from sugar-containing products should be consumed. However, if a participant experiences an adverse event, they are recommended to consume less than the calculated units and change to other S&SE products (e.g. avoid sugar-alcohols).
Example	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. 5 units per day or 35 units per week is then the maximum allowed intake of added sugar for this participant.	For a participant with an energy requirement of 9,000 kJ/d, 9.5 E% from sugar corresponds to 50 gram added sugar = 5 units. Ideally, this participant should consume 5 units per day or 35 units per week of S&SE containing products. One unit is equivalent to 1 unit in the sugar group (in weight or volume).

E%, energy percentage; S&SEs, sweeteners and sweetness enhancers.

Compliance:

Participants are required to record intake of all foods and drinks (pen and paper) for 4 days (3 weekdays and 1 weekend day) at months 0 and 12 with information on time, type/brand names, cooking and processing methods, weight or household measures. Daily average intake of energy, macro- and micro-nutrients will be calculated by national dietary software in the 4 intervention sites. From the food records, amount and intake of sugar and S&SEs in units from the products listed in Table 2 is also assessed at months 0 and 12. Furthermore, adults complete a food frequency questionnaire about intake of sweet products (sFFQ) during the past month and they collect 24-hour urine samples at months 0, 6, and 12. Urinary biomarkers of S&SEs (acesulfame-K, saccharin, sucralose, cyclamate, and steviol glycoronide) as well as fructose and sucrose will

be analysed by Wageningen University, the Netherlands, and urinary excretion of urea/nitrogen will be analysed locally. Based on the above listed data, the participants' compliance with the intervention diets will be assessed including associations between changes in outcomes e.g. body weight, energy consumption, and sugar consumption.

Data collection and outcomes

Including information meeting, screening, counselling sessions and CIDs, the trial consists of a minimum of 10 or 6 visits for adults and children, respectively. Data is collected according to standard operation procedures (SOPs) and Table 4 shows activities/data collection at each visit. Most data will be collected at months 0, 2, 6, and 12 where participants have fasted for a minimum of 10 hours and avoided intensive physical exercise, coffee, and smoking for 12 hours prior to the CIDs.

Table 4: Flow chart for adults (A) and children (C) (full sampling at months 0 and 12)

	Pre-screening	Information meeting	Screening	Baseline		*2-month period (CID1 to CID2)*		*10-month randomised intervention period (CID2 to CID4)*			1-year assessment
CID	-	-	-	CID1	-	-	CID2	-	CID3	-	CID4
Visit	-	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9
Month	-		-	0	0.5	1	2	4	6	9	12
Inclusion/exclusion criteria	A+C		A+C								
Signing Informed Consent		A+C[0]									
Med. hist., medication etc.			A+C								
Randomisation of the oldest family/household member			A								
Supervision/counselling				**A+C[1]**		A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**	A(+C)[2]	**A+C[1]**
Collection of LED products				**A[#]**	A[#]	A[#]	**A[#]**				
Body weight and height[4]			A+C	**A+C**	A[3]	A[3]	**A+C**	A[3]	**A+C**	A[3]	**A+C**
Waist and hip circumference				**A+C**			**A+C**		**A+C**		**A+C**
Body composition				**A+C[5]**			**A**				**A+C[5]**
Blood pressure and heart rate			A+C	**A+C**			**A+C**		**A+C**		**A+C**
Fasting blood samples			A[6]	**A+C**			**A+C[7]**		**A+C**		**A+C**
Adverse events and concomitant medication				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity (skin prick test)				**A**							**A**
24h urine collection (content of S&SEs)				**A**					**A**		**A**
Faecal spot sample				**A**			**A**		**A**		**A**
4-day dietary record				**A+C**							**A+C**
Questionnaires (electronic platforms):											
General background questionnaire				**A+C**							
Physical activity				**A+C**							**A+C**
Three factor eating questionnaire				**A+C**							**A+C**
Leeds food preference questionnaire				**A+C**			**A+C**		**A+C**		**A+C**
Allergenicity				**A+C**							**A+C**
Craving for sweet taste				**A**							**A**
Perception of S&SEs				**A**					**A[8]**		**A[8]**
Control of eating				**A**			**A**		**A**		**A**
Subjective appetite sensations				**A**			**A**		**A**		**A**
Sweet food frequency questionnaire (FFQ)				**A**							**A**
Diet satisfaction				**A**					**A**		**A**
Perception and evaluation of the intervention									**A**		**A**
Quality of life				**A**							**A**
Puberty				**C**					**C**		**C**

[#]Adults will collect LED products from the intervention site every 2nd or 3rd week during the 2-month period. At months 0.5 and 1.5 (optional) the adults will be weighed and have the opportunity to consult a dietician.
[0]For children, the informed consent is signed by the parents/guardians.
[1]Individual/family counselling is preferably scheduled at the same day as the CID.
[2]Group counselling, children participation is preferred, but not mandatory.
[3]Fasting is not required for this body weight measurement.
[4]Height is only measured at screening for adults.
[5]At University of Maastricht, body composition is not measured in children.
[6]At screening, fasting blood samples will be analysed at each intervention site. All other blood samples are analysed at the Central Laboratory (Bioiatriki).
[7]At University of Maastricht, a fasting blood sample is not drawn from children at CID2.
[8]A shorter version of the questionnaire is used at CID3-4.
A, adult; C, child; CID, clinical investigation day; LED, low-energy diet; S&SEs, sweeteners and sweetness enhancers.

Primary outcomes

This trial has 2 independent primary outcomes. The primary outcome for efficacy is 1-year change in body weight. The primary outcome for safety is 1-year change in gut microbiota composition associated with impaired health (e.g. change in microbial beta-diversity and composition). Both outcomes relate only to adults, and hence the required sample size was calculated for adults only.

Body weight

Body weight is measured to the nearest 0.1 kg using a digital scale with the participant wearing underwear/light clothes. Fasting body weight will be measured at screening and CIDs, however fasting is not required, when body weight is measured at other visits.

Gut microbiota

Gut microbiota composition will be assessed from faecal spot samples collected at home prior to all CIDs. Samples are immediately frozen (-20 °C) and later transported to the intervention site in cooling bags, whereafter they are stored at -80°C. Samples will be analysed targeting the V3-V4 regions of 16s rRNA genes by Illumina sequencing at Maastricht University, the Netherlands.

Secondary outcomes

Secondary outcomes include changes in anthropometry and body composition (for children, BMI-for-age z-score), risk factors for T2D and CVD, allergenicity, adverse events (AE), and concomitant medication. Additionally, some secondary outcomes will be assessed in adult sub-groups e.g. gut-brain signalling markers, postprandial energy expenditure and substrate oxidation, physical activity, liver fat, adipose tissue and lipid metabolism, brain reward, insulin sensitivity markers, and composition and functionality of the human gut microbiota in vitro. Furthermore, children's gut microbiota composition may be analysed depending on the final sample size (Table 5).

Anthropometry

Height is measured to the nearest 0.5 cm using a stadiometer at screening and for children at all CIDs. For adults, BMI is calculated as body weight (kg) / height^2 (m^2). For children, the WHO AnthroPlus software (www.who.int/tools/growth-reference-data-for-5to19-years/application-tools) is applied to calculate BMI-for-age percentile and z-score. Waist and hip circumferences are measured twice with a non-elastic tape measure on the skin to the nearest 0.5 cm, and the average is calculated. Waist circumference is measured halfway between the lowest rib and iliac crest during exhalation. Hip circumference is measured as the largest circumference in the area around the buttock. Dual-energy X-ray absorptiometry (DXA) scans are performed in underwear to assess body composition including fat percentage, fat mass and fat free mass.

Blood pressure and heart rate

After minimum 5 minutes rest in a sitting position, blood pressure (mmHg) and heart rate (beats per minute) are measured 3 times on the right arm with an automatically inflated cuff. An average is calculated from the last 2 measurements if the 2 measurements differ with ≤5 mmHg. If either the systolic or diastolic blood pressure differ by >5 mmHg, a 4th measurement is performed and the average calculated from the third and 4th measurement.

Blood samples

Fasting venous blood samples are drawn at all CIDs, except at month 2 for children at Maastricht due to Ethical concerns. Serum samples are collected for analyses of lipids (triglycerides, total, low-density lipoprotein, and high-density lipoprotein cholesterol), alanine aminotransferase, aspartate aminotransferase, insulin, C-reactive protein, and immunoglobulin E. Plasma is collected for glucose analysis and full blood for HbA1c analysis. All samples are stored locally at -80 ºC until shipment to the central lab at Bioiatriki S.A., Greece.

Skin prick test

For adults, a skin prick test is performed on the forearm. One drop of the allergens hazel, alder, birch, grass mix, artemisia absinthium, ragweed, alternaria, moulds mix, cat, dog,

dermatophagoides pteronyssinus and dermatophagoides mix as well as positive and negative control solutions are applied. The response is recorded after15 minutes.

Adverse events and concomitant medication

All AEs experienced after inclusion and during the trial are registered. At CIDs, the participant is asked if he/she has noticed any unfavourable events since the last CID. During the 10-month randomised intervention period, participants - regardless of intervention - are asked directly about certain AEs that may be related to consumption of S&SE i.e. gastrointestinal symptoms and headache. All medication necessary for the participants' health, not listed in the protocol exclusion criteria, may be continued during the trial. At CIDs, the participant is asked if he/she has taken any new medicine or has changed dosage of already registered medicine.

Other outcomes

Questionnaires are used to obtain information about sociodemographic characteristics such as education, occupation, household income etc., physical activity, quality of life, and to investigate subjective neuro-behavioural indices e.g. food preferences and preference for sweet taste, perception of S&SEs, cravings, subjective appetite sensations, and perception and evaluation of the 10-month randomised intervention period. Furthermore, puberty is assessed for children. All questionnaires are prepared in English and translated into local language. The majority of questionnaires will be delivered by a Questionnaire Delivery Platform (QDP) implemented by NetUnion, Switzerland. At baseline all questionnaires are completed at the intervention sites, but before other CIDs adults can complete those delivered by the QDP at home prior to the CID. Children always complete all questionnaires at the intervention site. Two questionnaires are always completed at the intervention site; 1 about perception of S&SEs (delivered by the Qualtrics platform via a weblink) and 1 about food rewards assessed by the Leeds Food Preference Questionnaire (E-prime software).

Sub-groups and sub-studies

Some of the secondary and other outcomes are collected in sub-studies and in sub-groups of different participants (Table 5).

Table 5: Secondary and other outcomes investigated in sub-studies and in sub-groups.

Outcome	Measurements and method	Participants	Time points of data collection			
			Baseline	After WL		After WM
			Month 0 CID1	Month 2 CID2	Month 6 CID3	Month 12 CID4
Sub-studies (include an intervention)						
Brain reward activity	Brain activity is measured by fMRI after consumption of a drink with sugar, S&SEs, water	Sub-study incl. a sub-group of adults in Maastricht	A	A		A
Postprandial responses (energy expenditure, substrate oxidation, blood biochemistry and appetite)	Indirect calorimetry, blood sampling, appetite sensation based on VAS and *ad libitum* energy intake after consumption of a drink with S&SE or water	Sub-study incl. a sub-group of adults in Copenhagen	A	A	A	
Sub-groups						
Physical activity	7-day measurements by accelerometer	Adults in Maastricht	A		A	A
Gut-brain signalling markers	Analyses of GLP-1, CCK, and ghrelin from fasting blood samples	Adults in Copenhagen and Maastricht	A	A	A	A
Liver fat	^{1}H-MRS	Sub-group of adults in Maastricht	A	A		A
Adipose tissue function and lipid metabolism	Adipocyte morphology, ex vivo lipolysis, gene and protein expression analyses of adipose tissue samples (biopsy)	Adults in Maastricht	A	A		A
Insulin sensitivity markers	Indices e.g. HOMA-IR, Matsuda index, Disposition index etc. calculated from a 7-point OGTT	Adults in Maastricht	A	A		A
Gut microbiota	16S rRNA illumine sequencing of faecal samples	Children in Maastricht	C		C	C
Composition and functionality of the human gut microbiota in vitro	Microbial metabolites, e.g. SCFA and 16S rRNA illumine sequencing of faecal samples	Sub-group of adults in Maastricht	A			

A, adults; C, children; CCK, cholecystokinin; CID, clinical investigation day; fMRI, functional Magnetic Resonance Imaging; GLP-1, glucagon-like peptide 1; ^{1}H-MRS, Proton Magnetic Resonance Spectroscopy; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance, OGTT, oral glucose tolerance test; SCFA, short chain fatty acids; S&SEs, sweeteners and sweetness enhancers; VAS, visual analogue scales; WL, weight loss, WM, weight maintenance.

Statistical methods

Sample size determination

The sample size calculation is based on adults for the 2 primary outcomes. For body weight, a clinically meaningful effect of 1.5 kg placebo subtracted body weight has previously been approved by the European Food Safety Authority.[24] Based on a similar trial [25] we estimated that a difference of 1.5 kg with a SD of ± 3.5 kg, a 90% power, a 2-sided α of 0.05, would require 231 completers. With an estimated drop-out of 30% a minimum of 330 adult participants should

be included. For change in gut microbiota, a ±10% change in 20 of the 50 most abundant operational taxonomic units (OTUs) with an alpha of <0.05%, would require 100 complete samples. The inclusion of at least 330 adults (approximately 25% of the participants per intervention site) is therefore also sufficient to detect possible changes in gut microbiota.

Statistical analysis plan

As part of the SWEET project a statistical analysis plan has been developed. For body weight, 1-year change between the 2 interventions will be analysed by analysis of covariance (ANCOVA) linear mixed model, with change in body weight as response; treatment group and relevant covariates e.g. age, gender, and BMI are fixed effects. Participant ID and intervention site are random (intercept) effects. Intention-to-treat principle will be applied on those completing the initial 2-month period. Additionally, complete-case analyses (all dropouts omitted) and per protocol analyses (only compliant participants) as well as analyses including additional covariate adjustments (e.g. energy density) and intermediate time points will be applied.

For gut microbiota, 1-year change in microbial diversity and microbial composition (relative abundance at phyla and genera level) will be analysed. Paired Wilcoxon test is used to study within intervention changes in relative abundance. Linear mixed models with Benjamin-Hochberg correction for multiple testing will be used for between-intervention comparisons.

Data will be presented with the use of standard descriptive statistics shown as mean (SD) or median (Q1:Q3) for normally and non-normally distributed data, respectively, and categorical data by percentages. Results will be presented as mean difference in changes ±SEM or 95% CIs and p-values when relevant. A statistical level of 0.05 will be applied and graphical models will be carried out to assess model assumptions. When relevant, transformation e.g. logarithm, will be applied or non-parametric statistical tests will be performed.

For secondary outcomes on continuous data, the main analysis will compare the 1-year mean change between the 2 treatment groups by use of the ANCOVA-type linear mixed model defined above without any multiplicity adjustment or imputation of missing values (i.e. available-case

analyses). Additional sensitivity analyses may be carried out as appropriate in the same way as for the primary outcome. Furthermore, analysis of repeated measures will be performed using linear mixed models including time×treatment interaction, time, and treatment effects, covariates (e.g. age, gender, BMI) as fixed effects, and participant ID and intervention sites as random effects. In case of significant time×treatment interaction, differences between treatments will be identified per time point. Mean changes will be compared between the groups using the estimated mean difference and approximate t-tests derived from the fitted linear mixed models (assuming a 2-sided alternative). For secondary categorical outcome (e.g. yes/no, 0/1/2, etc.) logistic or ordinal mixed effects model will be used, including the same fixed and random effects as the linear mixed models.

ETHICS AND DISSEMINATION

The RCT will be conducted in accordance with the Declaration of Helsinki[26] and this master protocol (version 3.0, 28-Oct-2020) is approved by the responsible national/regional committee in the 4 countries from where consent to all previous and future amendments to the protocol was and will be obtained. All adults receive the LED products free of charge. At Copenhagen, Navarra and Athens participants will not receive reimbursement for their participation. At Maastricht, travel expenses and financial compensation are provided to all eligible participants (125 Euros for adults without child(ren) and 250 Euros for 1 adult and 1 child + 80 Euros per extra family member).

There are no risks related to the dietary interventions, however discomfort may occur. The LED (not provided for children) contains all needed nutrients, but only little energy and therefore adults may experience headaches, dizziness, tiredness, and nausea particularly in the first few days. Constipation, stomach cramps or more profound nausea can occur and information on this is given before inclusion. However, allergic reactions to the LED are rare. The sugar and S&SEs intervention products are commercially available foods and drinks purchased in the supermarket and no adverse side effects are expected. However, changes in gastrointestinal symptoms (e.g.

bloating and excess gas production) may occur depending on the participant's habitual intakes of fibre and types of S&SEs e.g. sugar alcohols. At each intervention site, a physician can be consulted in case of medical uncertainties.

Some study procedures involve risks, but the procedures implemented are designed to minimize these. Drawing blood samples will seldom cause harm besides that associated with the insertion, however, children will be offered local anaesthetic Emla patches to reduce pain. A maximum of 80 ml and 125 ml of blood is drawn during the 1-year trial for children and adults, respectively. For children, this is less than 1 ml blood/kg body weight per donation, which is considered safe. Fertile women will be tested for pregnancy before DXA scanning and excluded from the trial if pregnant. The DXA scans will induce minor radiation (<0.010 mSv per scan). Scanning will be done 2 and 3 times during the 1-year trial for children and adults, respectively, and only 1 re-scan will be allowed per CID. The skin prick test, only performed in adults, is anticipated to cause very little discomfort. A positive reaction may cause itching, which will be treated with a salve. In very rare cases, a systemic anaphylactic reaction can occur and emergency equipment is in place.

For children, special attention is given to ensure that the child is not forced to participate by the adult family member. Furthermore, a child cannot remain included if the adult family member drops out or is excluded from the trial.

All participants will be insured against injury caused by their participation according to local legal requirements. The trial is monitored by the European Clinical Research Infrastructure Network (ECRIN) to ensure compliance with the protocol and SOPs. All trial-related information will be recorded, handled, and stored safely allowing accurate reporting, interpretation, and verification. All data will be collected in a central DataHub at Copenhagen from where pseudo-anonymised data can be requested until 2032 via a data sharing contract. From 2032, fully anonymised data can be transferred. Source data is collected on paper first or is entered directly into the electronic systems e.g. the QDP, the Qualtrics platform, and/or the Research Electronic

Data Capture (REDCap) tool hosted at the University of Copenhagen.[27,28] REDCap is a secure, web-based software platform designed to support data capture. Source data from DXA scans and analysis of biological material are registered on the device or related hardware, whereas the source data from dietary records (handwritten on paper) will be entered into a national dietary software program for analysis. The sponsor/investigator will provide direct access to source data/documents for inspection.

Results will be published in international peer-reviewed scientific journals regardless of whether the findings are positive, negative, or inconclusive.

DISCUSSION

The strengths of this RCT are the investigation of the long-term effects of S&SEs in the contexts of an *ad libitum* healthy diet not only including drinks, but also foods, compared to added sugar. The 10-month weight loss maintenance period - where the effects of S&SEs are investigated - is a very critical period. Thus based on previous research, individuals are expected to regain at least some of their lost body weight. [29,30] The long duration of the weight loss maintenance period is important when studying changes in body weight (1 of the 2 primary outcomes) as highlighted by the European Food Safety Authority (EFSA). [31] In 2020, the effect of S&SEs on body weight was extensively reviewed by Rogers & Appleton. [11] They identified 9 studies where effects of S&SEs, compared with sugar, were investigated in participants with overweight or obesity. Of these 9 studies, only 2 studies investigated effects of both foods and drinks [32,33] and 5 of the 9 studies investigated effects of a single S&SE. Furthermore, only 2 studies [34,35] had a duration of a minimum of 6 months, but none of these had the same weight loss and weight maintenance design as the current RCT. Thus, studies investigating the effect of S&SEs, compared to sugar, for weight loss maintenance are lacking. Other strengths are that the trial is conducted as a multicentre trial covering 4 European countries with both adults and children included. Furthermore, data will cover a broad range of measurements related to health (body weight management, risk factors for CVD, T2D, etc.) and safety (microbiology, allergy, adverse

events), appetite and food preferences, These will be measured at baseline, after a 2-month WL (adults) or 2-month weight stability (children) period, and after a 10-month intervention period. A trial limitation is that the number of included children was lower than initially planned, but this will not affect the 2 primary outcomes. Another limitation is that data related to energy and nutrient intakes (food records) are only collected at baseline and at months 12. However, urinary S&SE biomarkers collected at baseline, month 6 and month 12 will indicate if compliance decreases before month 12.

AUTHORS' CONTRIBUTIONS

The SWEET EU-project was initiated by JCGH, AR, and JH. The protocol for the SWEET intervention trial was written by LK, AR, and YM, with contribution from EEB and JAM. AR, YM, EEB, and JAM are principal investigators (PI) at the 4 intervention sites, where LK, SSHA, SN-C, KR and TCA are investigators. EJMF, GF, CEH, TL, and HM are responsible for specific methods, platforms, or analyses, and MdA is responsible for monitoring of the trial. LK and AR drafted the manuscript and YM, EEB, and JAM critically reviewed the manuscript. All authors read and approved the final manuscript.

FUNDING STATEMENT

The PI, AR, is also the sponsor (e-mail: ara@nexs.ku.dk, Phone: +45 21 30 69 12, Department of Nutrition, Exercise and Sports, University of Copenhagen, Rolighedsvej 26, 1958 Frederiksberg, Denmark). The trial is funded by the Horizon2020 program: *Sweeteners and sweetness enhancers: Impact on health, obesity, safety and sustainability* (acronym: SWEET, grant #774293) and funding covers salary for project personal, supplies, remuneration, and dissemination of results. The amount is deposited in a project account subject to audits/public revision.

COMPETING INTERESTS STATEMENT

AR has received honoraria from Unilever and the International Sweeteners Association. CEH's research centre provides consultancy to, and has received travel funds to present research results from organisations supported by food and drink companies. JCGH and JH have received project funds from the American Beverage Association. TL works for a company, NetUnion sarl, which has no conflict of interest in the study outcome.

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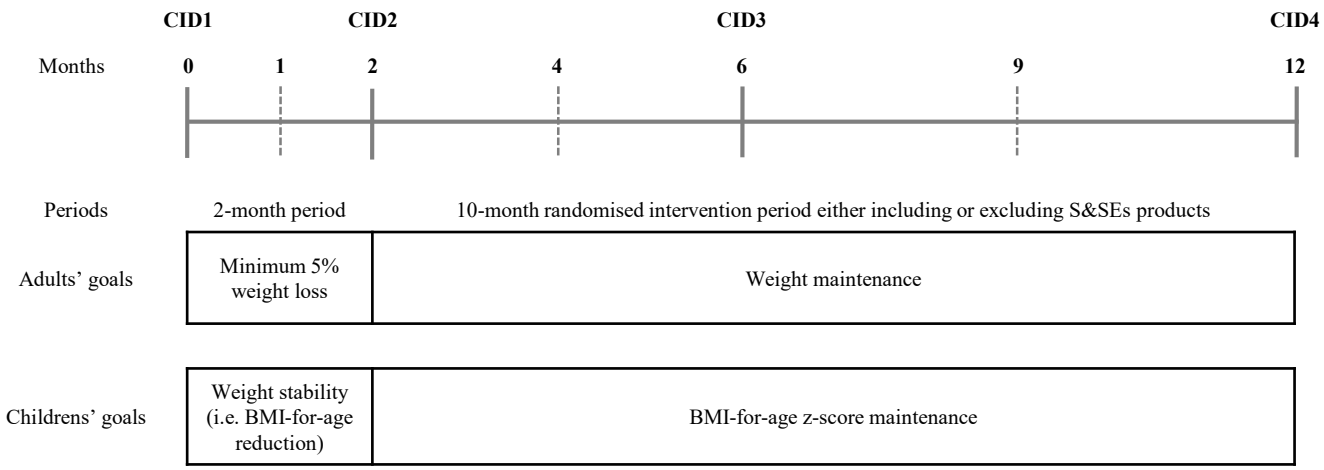
FIGURE LEGEND

Figure 1: Overall study design.
Solid lines are CIDs and dashed lines are dietary counselling sessions where non-fasting body weight of adults is measured. Additionally, LED products for adults will be collected from the intervention site every 2nd or 3rd week during the initial 2-month period.
BMI, body mass index; CID, clinical investigation day; S&SEs, sweeteners and sweetness enhancers.


CID1
CID2
CID3
CID4
Months
0
1
2
4
6
9
12
Periods
2-month period
10-month randomised intervention period either including or excluding S&SEs products
Adults' goals
Minimum 5% weight loss
Weight maintenance
Childrens' goals
Weight stability (i.e. BMI-for-age reduction)
BMI-for-age z-score maintenance

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
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	4
	2b	All items from the World Health Organization Trial Registration Data Set	NA
Protocol version	3	Date and version identifier	18
Funding	4	Sources and types of financial, material, and other support	21
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2+21
	5b	Name and contact information for the trial sponsor	21
	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	21
	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)	21
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	5
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6

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, noninferiority, exploratory)	6-7+9
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	6
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)	7-8+ Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	9-11 + Table 2+3
	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)	8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Table 1
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	14-17
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)	Figure 1 +Table 4
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	17
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
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	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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	9
Methods: Data collection, management, and analysis			
Data collection methods	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	12
	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	17
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	20
Statistical methods	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	17-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17-18
	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)	18

Methods: Monitoring			
Data monitoring	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	20
	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	NR
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	16
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NR
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)	18
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	7
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NR
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	19-20
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	20
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	18-19

Dissemination policy	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	4+20
	31b	Authorship eligibility guidelines and any intended use of professional writers	NR
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	20
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	The master version can be delivered by request
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	NR

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.