PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	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.
AUTHORS	Kjølbæk, Louise; Manios, Yannis; Blaak, Ellen; Martínez, Jose; Feskens, Edith; Finlayson, G; Andersen, Sabina; Reppas, Kyriakos; Navas-Carretero, Santiago; Adam, Tanja; Hodgkins, Charo; del Álamo, Marta; Lam, Tony; Moshoyiannis, Hariklia; Halford, Jason; Harrold, Joanne; Raben, Anne

VERSION 1 – REVIEW

REVIEWER	Feeney, Emma University College Dublin, Institute of Food and Health
REVIEW RETURNED	12-Apr-2022

GENERAL COMMENTS	This is a detailed study protocol for a timely and interesting study examining sweeteners and/or sweet enhancers, included as part of a weight maintenance strategy following weight loss with a Low Energy prescribed diet. The inclusion of children within the protocol is commendable and the format (as part of family) appears appropriate. Care has been taken to ensure that no one with either a diagnosed or self reported eating disorder will participate, and as BMI measures are not a requirement for the children, efforts have been made to ensure they are not made uncomfortable during their participation, although it will not be possible to completely remove that element. The authors have clearly stated the need for the study, and the sample size calculations to compare the healthy diet groups with and without sweeteners/ enhancers also seem appropriate. It is difficult to see how the authors plan to separate out the potential effect of differences in energy density between the healthy diets with and without sweeteners or sweetener enhancers, so this could be an additional consideration in the analysis. In the event that the SE/SEE diet is lower in energy, will the authors be able to attribute differences in dietary outcomes to energy reduction as opposed to sugar reduction? Compliance is checked at month 1 and 12 only, this could present a potential limitation if compliance falls off earlier for one diet vs the other. Although perhaps this will be picked up in the biomarkers that are collected more regularly. As the diets are ad libitum, will these 2

	timepoints be sufficient to adequately represent dietary differences between the intervention groups? It would be useful to provide clarity as to what was meant by 'safety markers' in the opening section and why gut microbiota is assessed for 'safety'. Regarding blood sampling (page 17), does this mean that blood samples are permitted to be taken for children at the other intervention sites? There is a small typo at Line 60, page 8 ‘to maintain’

REVIEWER	Page, Kathleen A. Univ So Calif
REVIEW RETURNED	Page, Kathleen A. Univ So Calif

GENERAL COMMENTS	This manuscript is a study protocol for a multicenter, parallel, randomized controlled trial on the effects of consuming low-calorie sweeteners & sweetener enhancers in combination with a low-sugar diet on weight loss maintenance and changes in the gut microbiota over 10 months among adults with overweight and obesity. A small sample of children with overweight and obesity are also included in exploratory analyses. Overall, the study design and protocol description are clear, but there are a few sections where the rationale and/or additional clarification may be helpful for readers as outlined below. (1) The basis for using changes in the gut microbiota composition as a “safety marker” of the treatment outcomes is not clear. It would be useful to provide information from prior studies that have established these changes in the gut microbiota as unsafe for health. (2) It would be helpful to clarify the definition of “sweetener and sweetness enhancers” because “sweetener” could refer to either caloric or non-caloric sweeteners. (3) The secondary outcomes did not list analyses for sex x treatment interactions. Are these planned? If so, then they should be stated in the analysis plan. (4) It would be good to include the background studies that provided the premise to support the 2-month energy restricted diet is sufficient time to achieve >=5% weight loss. How many subjects would be expected to fail, and thus would have to be excluded from the study? (5) Is there a requirement for children to achieve weight stability (which will reduce BMI-for-age z-score) after the 2-month run-in period? This should be clarified. (6) Secondary outcomes listed in the paper include gut-brain signaling markers, postprandial energy expenditure and substrate oxidation, liver fat, adipose tissue and lipid metabolism, brain reward (page 16). It is not clear from Table 4 which measures are being used to assess these outcomes. (7) What are the biomarkers that are being used to assess compliance with the S&SE diet from 24-hr urine samples?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Dr. Emma Feeney, University College Dublin

Comments to the Author:
This is a detailed study protocol for a timely and interesting study examining sweeteners and/or sweet enhancers, included as part of a weight maintenance strategy following weight loss with a Low Energy prescribed diet.
The inclusion of children within the protocol is commendable and the format (as part of family) appears appropriate. Care has been taken to ensure that no one with either a diagnosed or self reported eating disorder will participate, and as BMI measures are not a requirement for the children, efforts have been made to ensure they are not made uncomfortable during their participation, although it will not be possible to completely remove that element.
The authors have clearly stated the need for the study, and the sample size calculations to compare the healthy diet groups with and without sweeteners/ enhancers also seem appropriate.

It is difficult to see how the authors plan to separate out the potential effect of differences in energy density between the healthy diets with and without sweeteners or sweetener enhancers, so this could be an additional consideration in the analysis.

> RE: The energy content of the diet will be obtained from the Food records. At some, but not all sites, the total weight of consumed food and drinks will also be available. From these data, energy density of the diets in the two groups can be calculated.
> No change made in the manuscript.

In the event that the SE/SEE diet is lower in energy, will the authors be able to attribute differences in dietary outcomes to energy reduction as opposed to sugar reduction?

> RE: Yes, based on the Food records we will estimate both the total energy intake from the diet, the amount of consumed products (listed in Table 2), the amount of sugar from these products ("units" or grams) or the corresponding amount of "unit" from sweetened products. From all these measurements, we should be able to see if a reduced energy intake in the S&SE-group is caused by a reduced sugar intake.
> Furthermore, the adult participants complete a Food Frequency Questionnaire related to habitual intake of sweet products during the past months. From these, the habitual intake of sugar-products and sweetened-products can be estimated.
> We have added this extra information in the section "Compliance".

Compliance is checked at month 1 and 12 only, this could present a potential limitation if compliance falls off earlier for one diet vs the other. Although perhaps this will be picked up in the biomarkers that are collected more regularly. As the diets are ad libitum, will these 2 time points be sufficient to adequately represent dietary differences between the intervention groups?

> RE: We agree with the reviewer that these 2 time points are limited, but we collect urine samples at months 0, 6 and 12 from where some sweeteners (e.g acesulfame-K, saccharin, sucralose, cyclamate, and steviol glycoronide) as well as fructose and sucrose can be detected. In the SWEET consortium, another WP is working with the set up and validation of a method to assess the S&SE biomarker in the urine samples. The urinary biomarkers is an objective marker of S&SE intake, which is more reliable than a self-reported dietary registration - therefore also included more often. Furthermore, the majority of nutritional software programs do not provide information of content of S&SEs which is another limitation that explains why we decided to limit collection of this type of data and develop our own "unit-system".
> The information on S&SE biomarkers and time points has been added to the manuscript under the section "Compliance".

It would be useful to provide clarity as to what was meant by 'safety markers' in the opening section and why gut microbiota is assessed for 'safety'.

RE: Due to the fact that S&SEs are food additives the use and content of S&SEs in foods and drinks attract more attention with regard to toxicology and safety issues. Consumption of S&SEs has been claimed to results in different detrimental effects, and whether or not it is safe to consume (artificial) S&SE is highly debated in the media and the population. One of the safety aspects arise from studies observing changes in the gut microbiota composition and functionality of bacteria that have been associated with risk of diseases. Thisis why we use the term safety in the evaluation of the gut microbiota.
We have extended the description of gut microbiota evaluation in terms of safety in the introduction and added references to two recent reviews on this topic.

Regarding blood sampling (page 17), does this mean that blood samples are permitted to be taken for children at the other intervention sites?

RE: In Maastricht, their local ethical committee did not accepted the required blood volume. To reduce the blood volume, it was decided not to draw blood at CID2 (month 2).
Blood samples are drawn from the children at Maastricht at all other time point i.e. months 0, 6 and 12.
In all other intervention sites (Copenhagen, Athens and Navarra) blood samples are drawn from children at months 0, 2, 6 and 12.
A minor change in the Table 4 footnote has been made.

There is a small typo at Line 60, page 8 ‘to maintain’

RE: Thanks for notifying. It has been corrected.

Reviewer: 2
Dr. Kathleen A. Page, Univ So Calif

Comments to the Author:

This manuscript is a study protocol for a multicenter, parallel, randomized controlled trial on the effects of consuming low-calorie sweeteners & sweetener enhancers in combination with a low-sugar diet on weight loss maintenance and changes in the gut microbiota over 10 months among adults with overweight and obesity. A small sample of children with overweight and obesity are also included in exploratory analyses.

Overall, the study design and protocol description are clear, but there are a few sections where the rationale and/or additional clarification may be helpful for readers as outlined below.

(1) The basis for using changes in the gut microbiota composition as a “safety marker” of the treatment outcomes is not clear. It would be useful to provide information from prior studies that have established these changes in the gut microbiota as unsafe for health.

RE: Studies have indicated that consumption of S&SEs changes the gut microbiota composition and functionality, and that these changes have been associated with diseases and risk markers for diseases.
We have extended the description of gut microbiota evaluation in the introduction from the concrete example of one small poorly controlled human trial by adding references to two recent reviews on this topic and highlighting the general demand for controlled long-term human trials.

(2) It would be helpful to clarify the definition of “sweetener and sweetness enhancers” because “sweetener” could refer to either caloric or non-caloric sweeteners.

RE: In this project, we cover all types of sweeteners and sweetness enhancers meaning natural, artificial, non-caloric, low-caloric etc. This choice was made, because we wanted to have a very easy approach that potentially could be communicated to the public. Our participants are asked to purchase S&SE products in the super market and limiting the choice to specific types of sweeteners would be very difficult for the participants.
We have added this information in the section "*Ten-month period with S&SEs and sugar diets*"

(3) The secondary outcomes did not list analyses for sex x treatment interactions. Are these planned? If so, then they should be stated in the analysis plan.

RE: Thank you for the suggestion. Such analyses have not been planned, but we will certainly consider it.
No change has been made in the manuscript.

(4) It would be good to include the background studies that provided the premise to support the 2-month energy restricted diet is sufficient time to achieve >=5% weight loss. How many subjects would be expected to fail, and thus would have to be excluded from the study?

RE: Thank you for the suggestion. A 5% weight loss is generally accepted as clinically relevant. Based on several former weight loss maintenance studies conducted at our Department, we know that the average weight loss after a 8-week weight loss period on similar low energy diets (LED) will be 8-10% of initial body weight. Furthermore, it is our experience that few participants will fail and be excluded based on this criterion, but there will be other reasons for participants dropping out. This dropout and exclusion of participants has been taken into account in the overall expectation of a 30% dropout from the baseline (month 0) to the 1-year assessment.
In the manuscript, we have included a reference discussing the goal for a clinically meaningful weight loss.

(5) Is there a requirement for children to achieve weight stability (which will reduce BMI-for-age z-score) after the 2-month run-in period? This should be clarified.

RE: Thank you for pointing out that this was not completely clear.
We have added this sentence "For children, no weight criterion exists. Therefore, all children can continue into the WM period as long as their adult family member is included".

(6) Secondary outcomes listed in the paper include gut-brain signaling markers, postprandial energy expenditure and substrate oxidation, liver fat, adipose tissue and lipid metabolism, brain reward (page 16). It is not clear from Table 4 which measures are being used to assess these outcomes.

RE: We agree and have now included an extra Table (Table 5) which describes these measurements. We hope that the editor allows us to extend the manuscript in order to include this important information.

(7) What are the biomarkers that are being used to assess compliance with the S&SE diet from 24-hr urine samples?

RE: The five commonly consumed low caloric S&SEs, acesulfame-K, saccharin, sucralose, cyclamate, and steviol glycoronide can be detected from the urine. Furthermore, fructose and sucrose can be detected.
This information has been added to the manuscript under the section "Compliance".