Statistical Analysis Plan (SAP)

Sweet Tooth: Nature or Nurture? Role of Long-term Dietary Sweetness Exposure on Sweetness Preferences

Version 2024.02.07 (yyyy.mm.dd)

GENERAL INFORMATION	
Principal investigator	Dr. M (Monica) Mars Sensory Science and Eating Behaviour Wageningen University and Research Wageningen, the Netherlands
Core team members	Dr. Kees de Graaf (Project leader) [1] Dr. João Paulo (statistician) [1] Dr. Katherine Appleton [2] Eva Marija Čad [1] Claudia Tang [2] Leoné Pretorius [2] Hanne de Jong [1] [1]Division of Human Nutrition and Health, Wageningen University and Research, Wageningen, The Netherlands [2]Department of Psychology, Faculty of Science and Technology, Bournemouth University, Bournemouth, United Kingdom
Name of project	Sweet Tooth
ClinicalTrials.gov identifier	NCT04497974
Project Registration number	NL72134.081.19
Ethical review	METC-nr 20/02, METC-WU

INTRODUCTION	
Objectives	**Primary objective:** To assess the effect of a 6-month low, regular, and high dietary sweetness exposure on preference for sweet foods and beverages, and to compare these effects between the intervention groups. **Secondary Objective(s)**: To assess the effect of a 6-month low, regular, and high dietary sweetness expo- sure on taste intensity perception, behavioural outcomes: food choice and intake, sweet-liker type, food cravings, dietary taste preferences, dietary taste patterns; anthropometric outcomes: body composition, waist-hip circumference, body weight; and biochemical outcomes: glucose variability, and biomarkers related to CVD and diabetes.

STUDY METHODS	
Trial design	The Sweet Tooth study is a 6-month parallel randomized controlled trial with partial food provision. with three arms: 1. a regular sweet exposure diet (RSE, control) (n=60); 2. a low sweetness exposure diet (LSE) (n=60); and 3. a high sweetness exposure diet (HSE) (n=60).
Randomization	Data collected at the screening visit are used to assign participants to intervention groups. Matched groups are randomized to the interventions to minimize differences between intervention groups in these baseline characteristics. Based on sex (2 levels: male, female), age (3 levels: 18–34, 35–49, 50–65), Body Mass Index (BMI) (2 levels: 18.5–24.9, 25–30 kg/ m2) and sweet liker phenotype (3 levels: sweet liker, inverted U, sweet disliker), in a process of stratified randomization (2×3x2×3=36 strata). Treatment allocation is performed according to a computer-generated random schedule, at the ratio 1:1:1 to each of the three groups. Treatment allocation is performed by an independent person, not involved in the study outcome assessments or statistical analyses.
Sample size	The sample size calculation is based on our primary outcome: change in preference score from 0–6 months. Previous studies have established that it is possible to detect shifts in preferred concentration from preference tests. For example, looking at the study of Liem and de Graaf (2004) and the change in most preferred concentration, we can observe that mean ranking score for the sweet exposure group changed by around 0.4 and by around 0.6 for the sour exposure group from baseline to after exposure, which is around 10%. Therefore, the effect size of 0.1 was considered to be a relevant and meaningful effect size for our study and was used to estimate the sample size. We estimate that 147 participants are needed to detect an effect size of 0.1, assuming a parallel study with 3 groups, with two repeated measures (baseline vs 6-month, correlation between measures of 0.7), and a

	power of 80% at a significance level of 0.05. To account for a potential dropout of 20%, 180 individuals will be enrolled in the study.
Statistical interim analysis	No interim analyses will be carried out.
Timing of final analysis	Statistical analysis will be performed when the last subject leaves the follow up period.
Timing of outcome assessments	

Domain	Outcome to Be Measured	Data Collection Method	Baseline	Intervention			Follow-up	
				1Month	3 Months	6 Months	7 Months	10 Months
Food taste preference	Sweetness preferences	Rank-rating scale	✓	✓	✓	✓	✓	✓
	Saltiness preferences		✓	✓	✓	✓	✓	✓
Taste intensity perception	Sweet taste perception	100-unit VAS	✓	✓	✓	✓	✓	✓
	Salt taste perception		✓	✓	✓	✓	✓	✓
Behavioural outcomes	Food choice	Food choice from a buffet	✓	✓	✓	✓	✓	✓
	Food intake	Amount consumed from a buffet	✓	✓	✓	✓	✓	✓
	Sweet-liker type	100-point VAS	✓	✓	✓	✓	✓	✓
	Taste preferences	PrefQuest[a]	✓	✓	✓	✓	✓	✓
	Food cravings	CoEQ	✓	✓	✓	✓	✓	✓
	Dietary taste patterns	Taste FFQ[b]	✓	✓	✓	✓	✓	✓
Anthropometric outcomes	Body composition	DEXA	✓			✓		✓
	Waist-hip circumference	Measuring tape	✓	✓	✓	✓	✓	✓
	Weight	Digital scale	✓	✓	✓	✓	✓	✓
Biochemical outcomes	Biomarkers related to CVD and diabetes	Fasting blood sample	✓	✓	✓	✓	✓	✓
	Glucose homeostasis	Glucose sensor[c]	✓			✓		✓
Compliance	Biomarkers of compliance	Urine sample (24-h sample)	✓	✓	✓	✓	✓	✓
	Dietary intake	Online 24-h recall	✓	✓	✓	✓	✓	✓
Intervention Moderators	Physical activity	SQUASH	✓	✓	✓	✓	✓	✓
	Adverse events, medication use	Questionnaires[b], Study diet diary	✓	✓	✓	✓	✓	✓

CoEQ Control of eating questionnaire , *CVD* Cardiovascular disease, *DEXA* Dual-energy x-ray absorptiometry, *SQUASH* Short questionnaire to assess health enhancing physical activity , *VAS* Visual analogue scale

[a] Translated and Modified for the Dutch population based on Deglaire et al., 2012

[b] Developed for the Sweet tooth study based on methodology of Diewertje et al., 2016

[c] Only in a sub-set of participants ($n = 60$)

OUTCOMES		
Primary Outcome measure		
Outcome Measure	**Measure Description**	**Time Frame**
Change in preference score	Measured during preference testing, using Ranking on a scale methodology (scale anchored at 0: Dislike extremely; 50: Neither dislike or like; 100: Like extremely) in a series of test foods.	From month 0 to month 6.
Secondary Outcome measures		
Outcome Measure	**Measure Description**	**Time Frame**
Change in preference score.	Measured during preference testing, using Ranking on a scale methodology (scale anchored at 0: Dislike extremely; 50: Neither dislike or like; 100: Like extremely) in a series of test foods.	Measured at 0, 1, 3, 6, 7 and 10 months.
Difference in mean liking scores between familiar and unfamiliar foods.	Measured during preference testing, using Ranking on a scale methodology (scale anchored at 0: Dislike extremely; 50: Neither dislike or like; 100: Like extremely) in a series of test foods.	Measured at 0, 1, 3, 6, 7 and 10 months.
Change in sensory intensity scores.	Measured during sensory testing, using 100-unit Visual analogue scale (VAS), (anchored at 0: not sweet/salty at all; 100: Extremely sweet/salty) in a series of test foods.	Measured at 0, 1, 3, 6, 7 and 10 months.
Change in energy intake.	Measured during ad-libitum test meal in kcal.	Measured at 0, 1, 3, 6, 7 and 10 months.

Change in energy intake.	Measured during ad-libitum test meal in kJ.	Measured at 0, 1, 3, 6, 7 and 10 months.
Proportion of eaten sweet foods vs. foods from other taste modalities.	Measured during ad-libitum test meal in proportions.	Measured at 0, 1, 3, 6, 7 and 10 months
Sweet-liker status score.	Measured on a 100-unit VAS scale (anchored at 0: Dislike; 100: Like).	Measured at 0, 1, 3, 6, 7 and 10 months.
Food craving questionnaire scores.	Measured using the Control of eating questionnaire (CoEQ)	Measured at 0, 1, 3, 6, 7 and 10 months.
Taste preference questionnaire scores.	Measured using Taste Preference questionnaire (PrefQuest).	Measured at 0, 1, 3, 6, 7 and 10 months.
Dietary taste patterns.	Measured with the Taste food frequency questionnaire in frequency.	Measured at 0, 1, 3, 6, 7 and 10 months.
Dietary taste patterns.	Measured with the Taste food frequency questionnaire in % of energy coming from each taste cluster.	Measured at 0, 1, 3, 6, 7 and 10 months.
Dietary taste patterns.	Measured with the Taste food frequency questionnaire in % of food weight coming from each taste cluster.	Measured at 0, 1, 3, 6, 7 and 10 months.
Body weight.	Measured with a weighing scale in kg.	Measured at 0, 1, 2, 3, 4, 5, 6, 7 and 10 months.
Waist-to-hip ratio.	Measured using a stretch-resistant tape.	Measured at 0, 1, 3, 6, 7 and 10 months.
% of body fat mass and lean body mass (fat free mass).	Measured with a dual energy x-ray absorptiometry (DEXA).	Measured at 0, 6 and 10 months.
Variation in interstitial glucose levels.	Measured with glucose monitoring sensor (only measured in a subgroup, of 60 subjects, 20 per intervention arm).	Measured at 0, 6 and 10 months.
Change in fasting glucose, HbA1c, insulin, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides levels in blood.	Measured in blood in mmol/L.	Measured at 0, 1, 3, 6, 7 and 10 months.
Concentration of biomarkers in urine related to sugar, low and no calorie sweeteners, protein and salt intake.	Measured in urine in mg/d.	Measured at 0, 1, 3, 6, 7 and 10 months.
Intake levels of foods, food groups and macronutrients.	Measured with 24-hour recalls, in kcal/day.	Measured at 0, 1, 2, 3, 4, 5, 6, 7 and 10 months.
Other Outcome measures		
Outcome Measure	**Measure Description**	**Time Frame**
Adverse events.	Self-reported and monitored.	Measured at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10 months.
Gender.	Self-reported.	Assessed at month 0.
Height.	Measured with a stadiometer.	Assessed at month 0.
Physical activity level.	Measured with the Short Questionnaire to Assess Health enhancing physical activity (SQUASH).	Measured at 0, 1, 3, 6, 7 and 10 months.
Polymorphisms related to sweet taste perception.	Genes will be extracted from collected blood samples.	Assessed at month 0.
Age.	Self-reported.	Assessed at month 0.
Medicine usage.	Number and type of medicine used, self-reported.	Assessed at month 0.

STATISTICAL PRINCIPALS	
Principals and P-values	Prior to data analysis, normality of the data will be inspected. Non-normally distributed data will be transformed or analysed using non-parametric tests, if deemed necessary. Statistical significance is set at $p<0.05$.
Adherence and Protocol deviations	Adherence to diets will be assessed on group level with biomarkers of sweetener consumption in 24-h urine sample, and 24h recalls.

STATISTICAL ANALYSIS	
Analysis methods	Data will be analyzed in R. Based on findings of previous studies (Liem and de Graaf, 2004), we do not expect the data to be non-normally distributed. Descriptive statistics will be provided for each of the three intervention groups at baseline and will include demographic, dietary and lifestyle information. Continuous data will be summarized using means, SD/SE and 95% confidence intervals or median/geometric means and back-transformed 95% CI's if not normally distributed. Categorical variables will be summarized using counts and percentages. Analysis will be conducted on both an intention-to- treat (ITT) and a per-protocol basis. The ITT analysis will be the primary analysis. The per-protocol analysis (participants who finished the study), will help us determine whether the effects are the result of individuals adhering to the procedure and consuming the provided intervention foods. In the event of missing data due to drop-out, the outcome variables that have not been recorded will be treated as missing data. Unblinding will occur at the conclusion of the study to determine the effect of the intervention. Unblinding will take place in two steps: initially disclosing the allocation of individuals to their respective groups, without indicating intervention allocation. Only after the primary analyses have been conducted we will unblind the identity of each group. **Primary outcome:** To determine whether there is a shift in sweetness preference between baseline and month 6, statistical techniques appropriate for longitudinal data analysis, that is linear mixed effects models, will be used, with treatment (LSE, RSE and HSE), time (baseline, 1, 3, 6, 7 and 10-months) as fixed factors, the interaction between treatment x time outcome and participant number as a random factor. Our main interest is in the change in preference score between 0 and 6 months of any two intervention groups. The interaction effect will be evaluated to indicate if the change differed between the groups. If significant, the following predefined contrasts will be calculated: baseline vs. month 6 and LSE vs. RSE vs. HSE with all possible combinations. Subsequent, pairwise post-hoc tests will be Bonferroni adjusted.

	Other outcomes: Similar linear mixed effects models will be used to explore effects of sweet taste exposure on the other dependent measures over time (baseline, 1, 3, 6, 7 and 10-months). The interaction effect (intervention group x time) will be evaluated to indicate if the pattern of change of a certain outcome differed between the groups. If significant, the following predefined contrasts will be calculated: baseline vs. 1 vs. 3 vs. 6 vs. 7 vs. 10-months and LSE vs. RSE vs. HSE with all possible combinations. In light of the research in this area, we will explore differences between intervention groups and secondary outcome variables and explore related but separate questions of whether glucose variability, body composition, food cravings, blood biomarkers are related to sweetness exposure, age, gender, sweet liker status and BMI. By comparing group means of urine extraction of urinary sucrose, fructose, and LCSs between intervention groups, the level of compliance will be evaluated. Linear mixed effects models will be adjusted for covariates where appropriate and both unadjusted and adjusted models will be reported. If interaction effect between time and intervention are identified, Posthoc tests (with Bonferroni adjustments) will be applied to identify statistically significant differences.