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

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	MANAGEMENT, PUBLIC HEALTH, NUTRITION & DIETETICS

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4 **appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-**
5 **over, randomised, controlled trial in people with overweight/obesity. The SWEET**
6 **project.**
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40 Abstract

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

47 **Methods and Analysis:** A work package of the SWEET project, this study consists of 5
48 double-blind randomised cross-over trials which will be carried out at 5 sites across 4
49 European countries, aiming to have n=213. Five food matrices will be tested across 3
50 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends
51 and no added sugar). Participants (body mass index (BMI) 25-35 kg/m²; aged 18-60 years)
52 will consume each formulation for 14 days. The primary endpoint is composite appetite score
53 (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour
54 postprandial incremental area under the curve during clinical investigation days on day 1 and
55 14.

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

60 **Trial registration number:** Clinicaltrials.gov: NCT04633681

62 Strengths and Limitations of this study:

- 63 • The trial is the first of its kind to investigate the effects of acute and repeated exposure to
64 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety
65 of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary
66 (chocolate) and breakfast cereal.
- 67 • This trial includes a large range of outcomes across behaviour, physiology, and health
68 from persons living in Northern, Central and Southern Europe.
- 69 • The COVID-19 pandemic resulted in changes to the design of the studies in the trial.
70 Originally, all products were to be tested across 2 sites, but the reduced time frame means
71 this is not possible for some products.

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3 72 • Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be
4 73 reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will
5 74 be reported descriptively in these 2 studies where appropriate.
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10 76 **BACKGROUND AND RATIONALE**

11 77 The global increase in the prevalence of obesity and its associated diseases is driven by a
12 78 range of internal factors, involving genetic, behavioural, and metabolic determinants along
13 79 with permissive external factors from the physical, social, and political environment (1). Two
14 80 of the main behavioural drivers involve a diet too rich in energy and lack of sufficient
15 81 physical activity. Free sugar intake (derived from sugar added to foods and beverages by the
16 82 manufacturer or consumer) is one nutritional component that has gained focus because of its
17 83 low nutritional value (lack of vitamins, minerals or fibre) and its potential to add to the
18 84 overall energy density of diets, facilitating weight gain (2). In 2015, the World Health
19 85 Organisation (WHO) published a report with a specific focus on sugar intake and strongly
20 86 recommended that free sugar intake should constitute <10 % of total daily energy intake
21 87 (E%) and preferably <5 E% as a conditional recommendation (2). This is a recommendation
22 88 that is largely unaddressed in Europe (3). The replacement of free sugars with sweeteners and
23 89 sweetness enhancers (S&SEs) in food products is one method to reduce sugar intake while
24 90 maintaining acceptance and palatability of the diet. S&SEs have increasingly been employed
25 91 over recent years to reduce the energy and sugar content of foods; however, their impact on
26 92 appetite- and health-related outcomes is somewhat unclear (4).
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41 94 The effect of S&SEs on appetite is difficult to summarise due to the number of S&SEs
42 95 available and the different types of studies and comparisons used. It is likely that different
43 96 S&SE will have different modes of action and findings in one S&SE does not necessarily
44 97 translate to all S&SEs. A recent review comparing different S&SEs suggests that some have
45 98 the potential to enhance appetite, but these effects do not follow through to subsequent
46 99 energy intake (4). A recent meta-analysis detailed the impact of no- or low-energy sweetened
50 100 preloads compared to conventionally sweetened preloads on *ad libitum* energy intake. They
51 101 concluded that similar effects on energy intake were seen due to only partial compensation
52 102 being evident (although total energy intake was lower in the no- or low-energy sweetener)
53 103 (5). Furthermore, recent studies have highlighted that S&SEs may reduce sweet food
54 104 cravings and therefore reduce sugar intake (6) appetite and energy intake (7). Overall, there is
55 105 currently insufficient evidence to make a clear conclusion about the effect of S&SEs on
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3 106 appetite and energy intake. This is particularly true when discounting beverage studies, as
4
5 107 there is a lack of information regarding S&SEs in semi-solid and solid foods. Furthermore, it
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7 108 should be acknowledged that differences between acute and longer-term effects of S&SEs
8
9 109 may not be the same (8).

10
11 110

12 111 One of the reasons for the current partial understanding of the appetitive and metabolic
13
14 112 effects of S&SEs in humans is that different S&SEs are commonly assumed to have similar
15
16 113 behavioural effects (4, 9, 10). Only recently, one 12-week investigation of 4 distinct S&SEs
17
18 114 reported directionally dissimilar effects of saccharin compared to sucralose on body weight
19
20 115 (11). Indeed, the 11 S&SEs that are currently approved for use in the EU are chemically
21
22 116 heterogeneous and absorbed, metabolised, and excreted differently (12). Furthermore, most
23
24 117 investigations of the relationship between S&SE intake and health outcomes have used
25
26 118 beverages as the vehicle (13) ; these have recently been reviewed (14). Since the amount of
27
28 119 S&SEs in the food supply is increasing in response to consumer demand (15) and policy
29
30 120 (e.g., ‘sugar taxes’(16, 17); EU initiatives (18, 19)), there is a pressing need to examine the
31
32 121 appetite-related behavioural and metabolic consequences of consuming S&SEs particularly in
33
34 122 semi-solid and solid food matrices.

35 36 123 **AIMS AND OBJECTIVES**

37 124 The main objective of this study is to evaluate the acute (1-day) and repeated (14-day) effects
38
39 125 of 2 individual S&SEs and 2 S&SE blends in reformulated reduced or no added sugar food
40
41 126 products (using 2 modulations of S&SEs per matrix) on appetite and related behavioural,
42
43 127 metabolic and health outcomes in adult men and women with overweight or obesity.

44 128 The hypotheses are:

45
46 129 **H₁** Consumption of no added/reduced sugar products reformulated with S&SEs will result in
47
48 130 an altered incremental area under the curve (iAUC) appetite score, compared with the
49
50 131 sucrose-sweetened control product after repeated compared with acute consumption.

51 132

52
53 133 **H₂** There will be differences between the no added/reduced sugar and sucrose-sweetened
54
55 134 formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported
56
57 135 energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-
58
59 136 related (liver function and gastrointestinal (GI) side effects) outcomes.

60 137

138 TRIAL DESIGN

139 This study is part of the SWEET project (Sweeteners and sweetness enhancers: Impact on
140 health, obesity, safety and sustainability -Master protocol WP2 Phase 2 Version 2.6
141 29-MARCH-2022). It is a multicentre double-blind, randomised cross-over trial conducted
142 across 5 intervention sites in 4 countries, with 3 product formulations (sucrose-sweetened
143 control vs 2 individual S&SEs or S&SE blends) over 5 intervention product types (cake,
144 biscuits, yoghurt, chocolate, and breakfast cereal) aiming for a total of 213 completers:
145 Biscuit matrix n=52; yoghurt matrix n=54; chocolate matrix n=54; cereal matrix n=30; cake
146 matrix n=24. The protocol is reported as per the SPIRIT reporting guidelines (20).

147

148 *Sample Size Determination*

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

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

156 Secondary outcomes:

- 157 • Blood glucose - choosing 6.5 mmol/l as the blood glucose peak in the control condition, a
158 hypothesised 30% reduction for the S&SE conditions (4.55 mmol/L) and based on an
159 expected SD of 2.0 (23) an effect size (d_z) of 0.975 can be calculated using alpha of 0.01
160 (to account for multiple comparisons) and a two-tailed distribution. With these data the
161 minimum sample size is of 16 completers.
- 162 • Gut hormones - the minimum sample size for gut hormones was estimated based on values
163 for the pancreatic polypeptide (PP) (15' peak), according to published data (24). These
164 suggested a minimum of 30 completers per matrix were needed with 80% power, alpha
165 0.05 and a strong (0.9) effect size. It was estimated that this sample size would also suffice
166 to detect differences in plasma ghrelin in participants with overweight (25).

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

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

171 **STUDY SETTING**

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

179 **PATIENT AND PUBLIC INVOLVEMENT**

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

184 **ELIGIBILITY CRITERIA**

185 Male and female adults aged 18-60 years, with a BMI 25-35 kg/m² are eligible. Participants
186 are required to regularly consume sugar-containing foods and willing to consume sugar and
187 sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-
188 26) (26) score <20 and a short sweet food frequency questionnaire score ≥ 3 of 11, in addition
189 to rating the control product as $\geq 40\%$ on a 100-point liking VAS during the taste test and be
190 willing to consume the product during the duration of the trial. All exclusion criteria are
191 listed in **Supplemental Material 1**.

193 **INTERVENTION**

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

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2
3 199 the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be
4
5 200 followed by a wash out period of 14-21 days between formulations. During the at-home
6
7 201 periods, participants will consume a portion of the product at a time and place they choose
8
9 202 using a substitution strategy for similar energy/sweetness foods in their habitual diet. This
10
11 203 strategy is supported by advice and agreement from the research officer/dietitian. Compliance
12
13 204 will be monitored by an intervention booklet completed daily and by return of empty food
14
15 205 packaging. All food products are provided in the same blinded container/wrapping. The study
16
17 206 duration for each participant will be a minimum of 70 days (plus 7-14 days allowance for
18
19 207 extended washout to aid scheduling of CIDs).

208

209 **Recruitment and Screening**

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

229

230 **Randomisation and blinding**

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

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

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

242

243

244 **[Figure 1]**

245

246 **Clinical investigation days**

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

253

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

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3 266 something savoury and for something sweet on a validated 100-point VAS accessed via a PC
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5 267 or a tablet (30, 31). These measures will be completed on an electronic Questionnaire
6
7 268 Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be
8
9 269 measured using a culturally adapted version of the Leeds Food Preference Questionnaire
10
11 270 (LFPQ)(32) on a computer desktop. Appetite sensations measured by VAS will be repeated
12
13 271 after the LFPQ and before the researcher brings the blinded intervention product served with
14
15 272 200 mL of water. The participant will be instructed to take one bite, then answer questions
16
17 273 regarding sensory-specific satiety and expected satiety by VAS (33, 34). The participant will
18
19 274 be asked to consume the rest of the product over a period of 5-10 minutes, depending on the
20
21 275 time required to consume the matrix and asked to complete a set of appetite sensation
22
23 276 questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture
24
25 277 peak PP response (35) (yoghurt will be consumed faster than other products therefore blood
26
27 278 samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will
28
29 279 then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30,
30
31 280 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In
32
33 281 between measurements, participants will remain seated in a quiet area, free from food-related
34
35 282 sensory stimuli and read/listen to music/use a computer (provided there is no material with
36
37 283 reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete
38
39 284 the participant will be offered water or a snack before leaving the laboratory. Participants will
40
41 285 be reminded about the consumption of the products at home and that they will receive a
42
43 286 phone call the next day to complete a 24-hour diet recall and report any GI symptoms.
44
45 287 Following the end of the trial, participants will be debriefed if requested and offered the
46
47 288 chance to complete a survey about the conduct of the study.
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50 290
51 291 [Figure 2]
52 292
53 293

294 **Intervention products**

51 295 There will be 1 control product (sucrose-containing manufactured products) and 2 no
52
53 296 added/reduced sugar reformulated products based on the same food matrix - including 2
54
55 297 modulations of S&SE content (inclusion as individual S&SE or S&SE blends). The
56
57 298 reformulated products have a target of $\geq 30\%$ reduction in energy and/or sugar to achieve the
58
59 299 status of 'reduced sugar' by EU regulation No 1047/2012. This will not be possible in all
60
300 300 products, therefore 'no added sugar' will be applied to products who do not meet the criteria

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

305

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

308

309 Data Collection and Outcomes

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

311

312 **Table 1:** Data Collection and Timepoints for each CID

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

313 * Timepoints for PP are earlier for yoghurt study

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

320

321 Primary Outcome

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

326

327 Secondary Outcomes**328 Food preference and reward**

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

334

335 Food cravings

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

339

340 Energy intake

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

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

352

353 Expected satiety and sensory specific satiety

1
2
3 354 Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (33, 41) and
4
5 355 sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire
6
7 356 (34) after one bite and full consumption (10') of the product. Responses to both
8
9 357 questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will
10
11 358 be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).
12
13 359

14 360 *Other behavioural ratings*

15 361 Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will
16
17 362 be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).
18
19 363

20 364 *Biochemical measures*

21
22 365 Blood for plasma analyses will be centrifuged at 1500 g at 4°C for 10 minutes immediately
23
24 366 after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before
25
26 367 being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately
27
28 368 after collection. Plasma and serum aliquots will be stored at -80°C until shipment for
29
30 369 analyses to Bioaitriki Laboratories (central lab) in Athens, Greece.
31
32 370 Insulin concentrations will be determined by chemiluminescent microparticle immunoassay
33
34 371 (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system.
35
36 372 Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open
37
38 373 automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay
39
40 374 (Abbott) which consists of two separate concentration measurements: glycated haemoglobin
41
42 375 (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent
43
44 376 HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides
45
46 377 will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be
47
48 378 determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose
49
50 379 concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-
51
52 380 cholesterol will be determined by an accelerator selective detergent method (Ultra HDL
53
54 381 assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye
55
56 382 formation method (Direct LDL assay, Abbott). AST and ALT will be determined by
57
58 383 enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-
59
60 384 carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an
61
62 385 Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated
63
64 386 according to information provided in **Supplemental Material 5**.
65
66 387

388 ***Gastrointestinal (GI) side effects***

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

395

396 **STATISTICAL ANALYSIS PLAN**

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

415

416 ***Safety analysis***

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

419

420 **ETHICS AND MONITORING**

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3 421 Each intervention site will obtain ethical approval from their local ethical committee. All
4 422 study procedures will be conducted in accordance with the Helsinki Declaration and the study
5 423 protocol has been registered in a public database (clinicaltrials.gov NTC04633681;
6
7
8 424 **Supplemental Material 6**). To the extent relevant and reasonable International Council for
9
10 425 Harmonisation Good Clinical Practice (ICH-GCP) guidelines will be used, and standard
11
12 426 operating procedures (SOP) will be developed to facilitate the same performance and
13
14 427 compliance with the protocol in each centre. All personal data is handled confidentially and
15
16 428 stored in accordance with applicable law, GDPR and local laws (see Appendices). All
17
18 429 participants will receive written and oral information about the study and only trained study
19
20 430 personnel will provide information, monitor and attest signing of the informed consent form.
21
22 431 Where required, monitoring of intervention sites will be performed during the study by the
23
24 432 University of Navarra depending on local regulations.
25
26 433

434 **TRIAL STATUS**

27 435 Recruitment opened in May 2021 for the trial at the Leeds and Lyon intervention sites using the
28
29 436 biscuit matrix, with last participant last visit expected by June 2022. Recruitment for the trial
30
31 437 at Lyon using the cake matrix opened in February 2022. The trials at Liverpool, Copenhagen
32
33 438 and Pamplona will start recruiting in Spring 2022.
34
35 439

440 **PUBLICATION**

37 441 After completion of the study, the findings will be submitted for publication in an
38
39 442 international peer-reviewed scientific open access journal and other relevant media. Research
40
41 443 data from the trial will be deposited in an open access online research data archive (for
42
43 444 example Zenodo).
44
45 445

446 **FUNDING AND COMPETING INTERESTS STATEMENT**

48 447 The present study is funded by the Horizon 2020 program: Sweeteners and sweetness
49
50 448 enhancers: Impact on health, obesity, safety and sustainability (acronym: SWEET, grant no:
51
52 449 774293). The current study was initiated by Prof. G. Finlayson as part of the Work Package 2
53
54 450 of the SWEET project. The study receives funding from the Horizon2020 program (9 million
55
56 451 Euros) to cover salary for project personnel, supplies, remuneration and dissemination of
57
58 452 results. The amount is deposited in a project account subject to public revision. The funder
59
60 453 has no role in the study design, interpretation of data or publication of material.

1
2
3 454 JCGH, JAH and CAH and are in receipt of research funding from the American Beverage
4
5 455 Association. CAH has received honoraria from the International Sweeteners Association.
6
7 456 ARA has received honoraria from Unilever and the International Sweeteners Association.
8
9 457 CH's research centre provides consultancy to and has received travel funds to present
10
11 458 research results from organisations supported by food and drink companies. CS is a paid
12
13 459 employee of Cargill, Inc.

14 15 460 **AUTHORS' CONTRIBUTIONS**

16
17
18
19 461 **Joint first authors:** Equal contribution from Catherine Gibbons and Beverley O'Hara

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29 465 Wilton, Louise Kjølbaek, Anne Raben and Charo Hodgkins

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32 466 **With contribution from SWEET Work Pack 2 Phase 2 study partners:** Cécile Rannou,
33
34 467 Julie-Anne Nazare, Maud Alligier, Dominic O'Connor, Ellen E Blaak, Jason Halford, Mie
35
36 468 Normand, Edith Feskens and Hariklia Moshoyiannis

37 38 39 470 **REFERENCES**

- 40
41 471 1. Mitchell NS, Catenacci VA, Wyatt HR, Hill JO. Obesity: overview of an epidemic.
42
43 472 Psychiatr Clin North Am. 2011;34(4):717-32.
- 44
45 473 2. World Health Organisation. Guideline: Sugars intake for adults and children. Geneva:
46
47 474 World Health Organisation; 2015.
- 47
48 475 3. Azaïs-Braesco V, Sluik D, Maillot M, Kok F, Moreno LA. A review of total & added
49
50 476 sugar intakes and dietary sources in Europe. Nutr J. 2017;16(1):6.
- 51
52 477 4. O'Connor D, Pang M, Castelnuovo G, Finlayson G, Blaak E, Gibbons C, et al. A
53
54 478 rational review on the effects of sweeteners and sweetness enhancers on appetite, food
55
56 479 reward and metabolic/adiposity outcomes in adults. Food Funct. 2021;12(2):442-65.
- 56
57 480 5. Lee HY, Jack M, Poon T, Noori D, Venditti C, Hamamji S, et al. Effects of
58
59 481 Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners
- 60

- 1
2
3 482 on Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled
4 483 Human Intervention Studies. *Adv Nutr.* 2021;12(4):1481-99.
- 5
6 484 6. Rogers PJ, Ferriday D, Irani B, Hei Hoi JK, England CY, Bajwa KK, et al. Sweet
7 485 satiation: Acute effects of consumption of sweet drinks on appetite for and intake of sweet
8 486 and non-sweet foods. *Appetite.* 2020;149:104631.
- 9
10
11 487 7. Stamatakis NS, Crooks B, Ahmed A, McLaughlin JT. Effects of the Daily
12 488 Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A
13 489 Randomised Open-Label 12-Week Trial in Healthy Adults. *Nutrients.* 2020;12(10).
- 14
15 490 8. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-
16 491 energy sweetener consumption affect energy intake and body weight? A systematic review,
17 492 including meta-analyses, of the evidence from human and animal studies. *International*
18 493 *journal of obesity (2005).* 2016;40(3):381-94.
- 19
20 494 9. Hunter SR, Reister EJ, Cheon E, Mattes RD. Low Calorie Sweeteners Differ in Their
21 495 Physiological Effects in Humans. *Nutrients.* 2019;11(11):2717.
- 22
23 496 10. Pang MD, Goossens GH, Blaak EE. The Impact of Artificial Sweeteners on Body
24 497 Weight Control and Glucose Homeostasis. *Frontiers in nutrition.* 2021;7:598340-.
- 25
26 498 11. Higgins KA, Mattes RD. A randomized controlled trial contrasting the effects of 4
27 499 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *The*
28 500 *American Journal of Clinical Nutrition.* 2019;109(5):1288-301.
- 29
30 501 12. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate
31 502 of low-calorie sweeteners. *Nutr Rev.* 2016;74(11):670-89.
- 32
33 503 13. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing
34 504 metabolic derangements. *Trends Endocrinol Metab.* 2013;24(9):431-41.
- 35
36 505 14. McGlynn ND, Khan TA, Wang L, Zhang R, Chiavaroli L, Au-Yeung F, et al.
37 506 Association of Low- and No-Calorie Sweetened Beverages as a Replacement for Sugar-
38 507 Sweetened Beverages With Body Weight and Cardiometabolic Risk: A Systematic Review
39 508 and Meta-analysis. *JAMA Network Open.* 2022;5(3):e222092-e.
- 40
41 509 15. Nunn R, Young L, Ni Mhurchu C. Prevalence and Types of Non-Nutritive
42 510 Sweeteners in the New Zealand Food Supply, 2013 and 2019. *Nutrients.* 2021;13(9).
- 43
44 511 16. Ng SW, Colchero MA, White M. How should we evaluate sweetened beverage tax
45 512 policies? A review of worldwide experience. *BMC Public Health.* 2021;21(1):1941.
- 46
47 513 17. Russell C, Grimes C, Baker P, Sievert K, Lawrence MA. The drivers, trends and
48 514 dietary impacts of non-nutritive sweeteners in the food supply: a narrative review. *Nutrition*
49 515 *Research Reviews.* 2021;34(2):185-208.

- 1
2
3 516 18. Webster J. Working paper on product reformulation and portion size. Brussels: EU
4 517 Platform on diet, physical activity and health; 2009.
- 5
6 518 19. World Health Organisation. Sugar-sweetened beverage taxes in the WHO European
7 519 Region: success through lessons learned and challenges faced. Copenhagen. WHO Regional
8 520 Office for Europe Licence: CC BY-NC-SA 3.0 IGO; 2022. Contract No.:
9 521 WHO/EURO:2022-4781-44544-6381.
- 10
11 522 20. Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT
12 523 2013 explanation and elaboration: guidance for protocols of clinical trials. *BMJ : British
13 524 Medical Journal.* 2013;346:e7586.
- 14
15 525 21. Almiron-Roig E, Green H, Virgili R, Aeschlimann JM, Moser M, Erkner A.
16 526 Validation of a new hand-held electronic appetite rating system against the pen and paper
17 527 method. *Appetite.* 2009;53(3):465-8.
- 18
19 528 22. Jones BK, M. G. Design and Analysis of Cross-Over Trials. 3rd Edition ed. Boca
20 529 Raton, Florida: CRC Press; 2015.
- 21
22 530 23. Green MW, Taylor MA, Elliman NA, Rhodes O. Placebo expectancy effects in the
23 531 relationship between glucose and cognition. *Br J Nutr.* 2001;86(2):173-9.
- 24
25 532 24. Yeomans MR, Re R, Wickham M, Lundholm H, Chambers L. Beyond expectations:
26 533 the physiological basis of sensory enhancement of satiety. *Int J Obes (Lond).*
27 534 2016;40(11):1693-8.
- 28
29 535 25. Bowen J, Noakes M, Trenerry C, Clifton PM. Energy intake, ghrelin, and
30 536 cholecystokinin after different carbohydrate and protein preloads in overweight men. *J Clin
31 537 Endocrinol Metab.* 2006;91(4):1477-83.
- 32
33 538 26. Garner DM, Garfinkel PE. The Eating Attitudes Test: an index of the symptoms of
34 539 anorexia nervosa. *Psychol Med.* 1979;9(2):273-9.
- 35
36 540 27. Masic U, Harrold JA, Christiansen P, Cuthbertson DJ, Hardman CA, Robinson E, et
37 541 al. EffectS of non-nutritive sWeetened beverages on appetITe during aCtive weigHt loss
38 542 (SWITCH): Protocol for a randomized, controlled trial assessing the effects of non-nutritive
39 543 sweetened beverages compared to water during a 12-week weight loss period and a follow up
40 544 weight maintenance period. *Contemp Clin Trials.* 2017;53:80-8.
- 41
42 545 28. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc
43 546 Sport.* 2000;71 Suppl 2:114-20.
- 44
45 547 29. Dalton M, Finlayson G, Hill A, Blundell J. Preliminary validation and principal
46 548 components analysis of the Control of Eating Questionnaire (CoEQ) for the experience of
47 549 food craving. *European Journal of Clinical Nutrition.* 2015;69(12):1313-7.

- 1
2
3 550 30. Brunger L, Smith A, Re R, Wickham M, Philippides A, Watten P, et al. Validation of
4 551 an iPad visual analogue rating system for assessing appetite and satiety. *Appetite*.
5 552 2015;84:259-63.
6
7
8 553 31. Gibbons C, Caudwell P, Finlayson G, King N, Blundell J. Validation of a new hand-
9 554 held electronic data capture method for continuous monitoring of subjective appetite
10 555 sensations. *Int J Behav Nutr Phys Act*. 2011;8:57.
11
12 556 32. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit
13 557 liking and wanting for food: implications for appetite control. *Appetite*. 2008;50(1):120-7.
14
15 558 33. Forde CG, Almiron-Roig E, Brunstrom JM. Expected Satiety: Application to Weight
16 559 Management and Understanding Energy Selection in Humans. *Current obesity reports*.
17 560 2015;4(1):131-40.
18
19 561 34. Snoek HM, Huntjens L, Van Gemert LJ, De Graaf C, Weenen H. Sensory-specific
20 562 satiety in obese and normal-weight women. *Am J Clin Nutr*. 2004;80(4):823-31.
21
22 563 35. Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine Cephalic Phase
23 564 Responses to Food Cues: A Systematic Review. *Adv Nutr*. 2020;11(5):1364-83.
24
25 565 36. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association
26 566 between the effect of carbohydrates on blood glucose and subsequent short-term food intake
27 567 in young men. *The American Journal of Clinical Nutrition*. 2002;76(5):1023-30.
28
29 568 37. Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite
30 569 control: Methodological aspects of the evaluation of foods. *Obesity Reviews*.
31 570 2010;11(3):251-70.
32
33 571 38. Steinfeldt L, Anand J, Murayi T. Food Reporting Patterns in the USDA Automated
34 572 Multiple-Pass Method. *Procedia Food Science*. 2013;2:145-56.
35
36 573 39. Australian Health Survey. Food Model Booklet. In: Statistics ABo, editor. Belconnen
37 574 ACT2010.
38
39 575 40. Zandstra EH, Mathey MF, Graaf C, van Staveren WA. Short-term regulation of food
40 576 intake in children, young adults and the elderly. *Eur J Clin Nutr*. 2000;54(3):239-46.
41
42 577 41. Brunstrom JM, Shakeshaft NG, Scott-Samuel NE. Measuring 'expected satiety' in a
43 578 range of common foods using a method of constant stimuli. *Appetite*. 2008;51(3):604-14.
44
45 579 42. O'Donnell LJ, Virjee J, Heaton KW. Detection of pseudodiarrhoea by simple clinical
46 580 assessment of intestinal transit rate. *BMJ (Clinical research ed)*. 1990;300(6722):439-40.
47
48 581 43. Svedlund J, Sjödin I, Dotevall G. GSRs--a clinical rating scale for gastrointestinal
49 582 symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci*.
50 583 1988;33(2):129-34.

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43
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46
47
48
49
50
51
52
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56
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584 44. Wasserstein RL, Lazar NA. The ASA Statement on p-Values: Context, Process, and
585 Purpose. The American Statistician. 2016;70(2):129-33.

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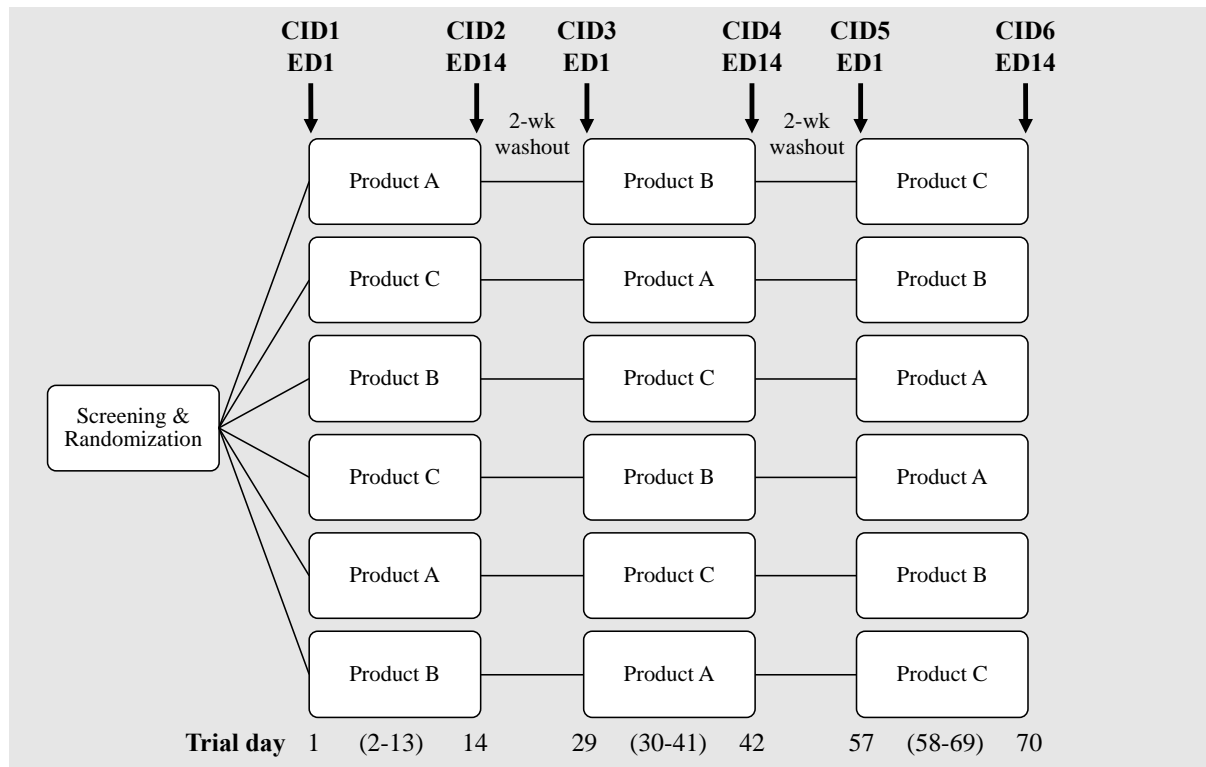


Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to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

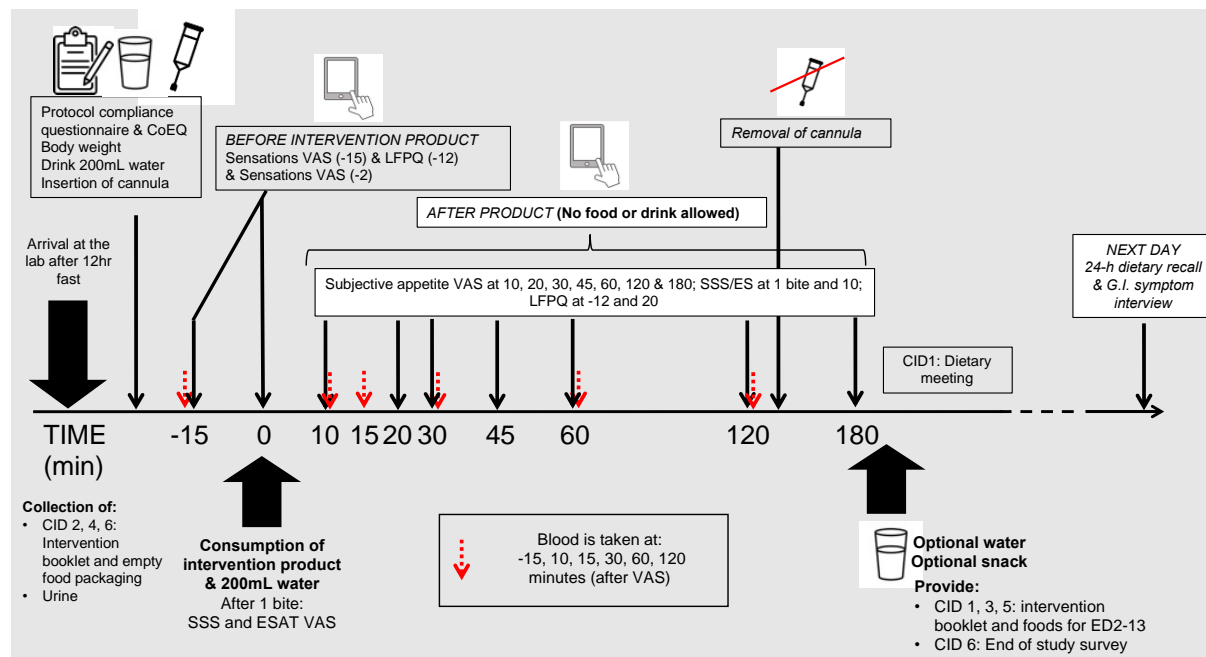


Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix.

CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.

Supplemental Material 1: Exclusion Criteria

General Criteria

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

Medical conditions as known by the person

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

Medication

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2
3 • Use currently or within the previous 3 months of prescription or over the counter
4 medication that has the potential of affecting appetite, satiety, or body weight incl. food
5 supplements.

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

- 10 • Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e.,
11 the medication is allowed if the participant has been on a stable dose for at least 3 months).
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Supplemental Material 2: Product Ingredients List

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

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

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

For peer review only

Supplementary Material 3 – Energy Intake Calculation

Percent energy compensation (%EC) was derived from the dietary recall data as previously reported by Zandstra et al.¹, and Almiron-Roig et al². Briefly, %EC was calculated as:

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

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

1. Zandstra, E. H., Mathey, M. F., Graaf, C., & van Staveren, W. A. (2000). Short-term regulation of food intake in children, young adults and the elderly. *European Journal of Clinical Nutrition*, 54(3), 239-246. <http://www.ncbi.nlm.nih.gov/pubmed/10713747>
2. Almiron-Roig, E., Palla, L., Guest, K., Ricchiutu, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews*, 71(7), 458-473. [https://doi.org/10.1111.nure.12048](https://doi.org/10.1111/nure.12048)

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2
3 **Supplemental Material 4: Questions used to assess product taste text, subjective**
4 **appetite sensations, sensory specific satiety and expected satiety**
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6
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8 **Food Taste Test (FTT) (Conducted at screening)**
9

10
11 **Screen 1**
12

13 You will now be presented with a food that we will ask you to evaluate. Please follow the
14 instructions as they appear on the screen.
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16
17
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20

21 -----
22 **Screen 2**
23

- 24
25 1. Take a mouthful of the food provided.
26 2. Chew while counting to 5.
27 3. Swallow.
28 4. Answer the question by moving the arrow to the left or to the right.
29
30

31 **How pleasant was this food?**
32
33 Not at all pleasant Extremely
34 pleasant
35
36
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40 -----
41 **Screen 3**
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43 Thank you. This is the end of the taste test.
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45 Please call the investigator after submitting your answer.
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Subjective Appetite Questions (used during all CIDs)

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

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

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

After 1 bite:

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

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

At 10 minutes:

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

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

After 1 bite:

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

At 10 minutes after full consumption and after SSS rating:

How much will this food stop you from feeling hungry between meals?

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

Calculation of Fatty Liver Index:

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

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

Calculation of Triglyceride Glucose Index:

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

$$\text{TyG index} = \text{Ln} [(\text{fasting triglycerides}) (\text{mg/dL}) \times \text{fasting glucose} (\text{mg/dL})] / 2$$

1. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterology*, 6(1), 33. <https://doi.org/10.1186/1471-230X-6-33>
2. Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, 6(4), 299–304. <https://doi.org/10.1089/met.2008.0034>

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

Supplemental Material 6: Trial Registration Data Set

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

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2	
3	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering
4	medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable
5	dose for at least 3 months)
6	Interventional
7	Allocation: Randomised. Double-blind, within-subjects, cross-over trial
8	
9	Study type
10	Primary purpose: Evaluation
11	Phase: N/A
12	Date of first enrolment
13	April 2021
14	Target sample size
15	213
16	Recruitment status
17	Recruiting
18	Primary outcome(s)
19	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
20	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of
21	Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose
22	Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers:
23	Glucagon-like peptide-1 (GLP-1) Incremental area under the curve for blood GLP-1 concentrations in response to each product
24	(120 min post intake); Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product
25	(120 min post intake).
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Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

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

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

1	Roles and	#5b	Name and contact information for the trial	15
2	responsibilities:		sponsor	
3	sponsor contact			
4	information			
5				
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7				
8	Roles and	#5c	Role of study sponsor and funders, if any, in	15
9	responsibilities:		study design; collection, management, analysis,	
10	sponsor and funder		and interpretation of data; writing of the report;	
11			and the decision to submit the report for	
12			publication, including whether they will have	
13			ultimate authority over any of these activities	
14				
15				
16				
17	Roles and	#5d	Composition, roles, and responsibilities of the	7 and 15
18	responsibilities:		coordinating centre, steering committee,	
19	committees		endpoint adjudication committee, data	
20			management team, and other individuals or	
21			groups overseeing the trial, if applicable (see	
22			Item 21a for data monitoring committee)	
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27	Introduction			
28				
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30	Background and	#6a	Description of research question and justification	4
31	rationale		for undertaking the trial, including summary of	
32			relevant studies (published and unpublished)	
33			examining benefits and harms for each	
34			intervention	
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38	Background and	#6b	Explanation for choice of comparators	4
39	rationale: choice of			
40	comparators			
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42				
43	Objectives	#7	Specific objectives or hypotheses	5
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46	Trial design	#8	Description of trial design including type of trial	6
47			(eg, parallel group, crossover, factorial, single	
48			group), allocation ratio, and framework (eg,	
49			superiority, equivalence, non-inferiority,	
50			exploratory)	
51				
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**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
2				
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
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14	Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
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20	Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
21				
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27	Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
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34	Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
35				
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
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1	Sample size	#14	Estimated number of participants needed to	6
2			achieve study objectives and how it was	
3			determined, including clinical and statistical	
4			assumptions supporting any sample size	
5			calculations	
6				
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9	Recruitment	#15	Strategies for achieving adequate participant	8
10			enrolment to reach target sample size	
11				
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13	Methods:			
14	Assignment of			
15	interventions (for			
16	controlled trials)			
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20	Allocation: sequence	#16a	Method of generating the allocation sequence	8-9
21	generation		(eg, computer-generated random numbers), and	
22			list of any factors for stratification. To reduce	
23			predictability of a random sequence, details of	
24			any planned restriction (eg, blocking) should be	
25			provided in a separate document that is	
26			unavailable to those who enrol participants or	
27			assign interventions	
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33	Allocation	#16b	Mechanism of implementing the allocation	9
34	concealment		sequence (eg, central telephone; sequentially	
35	mechanism		numbered, opaque, sealed envelopes),	
36			describing any steps to conceal the sequence	
37			until interventions are assigned	
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41	Allocation:	#16c	Who will generate the allocation sequence, who	8
42	implementation		will enrol participants, and who will assign	
43			participants to interventions	
44				
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46	Blinding (masking)	#17a	Who will be blinded after assignment to	9
47			interventions (eg, trial participants, care	
48			providers, outcome assessors, data analysts),	
49			and how	
50				
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52				
53	Blinding (masking):	#17b	If blinded, circumstances under which unblinding	N/A: No safety/critical
54	emergency		is permissible, and procedure for revealing a	efficacy issues present
55	unblinding		participant's allocated intervention during the trial	
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1 **Methods: Data**
 2 **collection,**
 3 **management, and**
 4 **analysis**
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8	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
9			15; Supplemental Material 4
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22	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
23			N/A Data from participants who discontinue/ deviate from protocol not collected
24			
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30	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
31			15; appendices
32			
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40	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
41			14
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47	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
48			N/A, no analysis included
49			
50			
51	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
52			N/A, no analysis included
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Methods:**Monitoring**

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5	Data monitoring:	#21a	Composition of data monitoring committee
6	formal committee		(DMC); summary of its role and reporting
7			structure; statement of whether it is independent
8			from the sponsor and competing interests; and
9			reference to where further details about its
10			charter can be found, if not in the protocol.
11			Alternatively, an explanation of why a DMC is
12			not needed
13			
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18	Data monitoring:	#21b	Description of any interim analyses and stopping
19	interim analysis		guidelines, including who will have access to
20			these interim results and make the final decision
21			to terminate the trial
22			
23			
24	Harms	#22	Plans for collecting, assessing, reporting, and
25			managing solicited and spontaneously reported
26			adverse events and other unintended effects of
27			trial interventions or trial conduct
28			
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31	Auditing	#23	Frequency and procedures for auditing trial
32			conduct, if any, and whether the process will be
33			independent from investigators and the sponsor
34			
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37	Ethics and		
38	dissemination		
39			
40	Research ethics	#24	Plans for seeking research ethics committee /
41	approval		institutional review board (REC / IRB) approval
42			
43			
44	Protocol	#25	Plans for communicating important protocol
45	amendments		modifications (eg, changes to eligibility criteria,
46			outcomes, analyses) to relevant parties (eg,
47			investigators, REC / IRBs, trial participants, trial
48			registries, journals, regulators)
49			
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52	Consent or assent	#26a	Who will obtain informed consent or assent from
53			potential trial participants or authorised
54			surrogates, and how (see Item 32)
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N/A: No safety/critical efficacy issues present

N/A: No safety/critical efficacy issues present

N/A: No safety/critical efficacy issues present

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N/A

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1	Consent or assent:	#26b	Additional consent provisions for collection and	N/A
2	ancillary studies		use of participant data and biological specimens	
3			in ancillary studies, if applicable	
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6	Confidentiality	#27	How personal information about potential and	Appendices
7			enrolled participants will be collected, shared,	
8			and maintained in order to protect confidentiality	
9			before, during, and after the trial	
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13	Declaration of	#28	Financial and other competing interests for	15
14	interests		principal investigators for the overall trial and	
15			each study site	
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18	Data access	#29	Statement of who will have access to the final	15
19			trial dataset, and disclosure of contractual	
20			agreements that limit such access for	
21			investigators	
22				
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25	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial	N/A: No safety/critical
26	trial care		care, and for compensation to those who suffer	efficacy issues present
27			harm from trial participation	
28				
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31	Dissemination	#31a	Plans for investigators and sponsor to	15
32	policy: trial results		communicate trial results to participants,	
33			healthcare professionals, the public, and other	
34			relevant groups (eg, via publication, reporting in	
35			results databases, or other data sharing	
36			arrangements), including any publication	
37			restrictions	
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42	Dissemination	#31b	Authorship eligibility guidelines and any intended	16
43	policy: authorship		use of professional writers	
44				
45				
46	Dissemination	#31c	Plans, if any, for granting public access to the full	15
47	policy: reproducible		protocol, participant-level dataset, and statistical	
48	research		code	
49				
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51	Appendices			
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54	Informed consent	#32	Model consent form and other related	Appendices
55	materials		documentation given to participants and	
56			authorised surrogates	
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1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and 12
2 storage of biological specimens for genetic or
3 molecular analysis in the current trial and for
4 future use in ancillary studies, if applicable
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7

8 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
9 Commons Attribution License CC-BY-NC. This checklist can be completed online using
10 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
11 [Penelope.ai](#)
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BMJ Open

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

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Primary Subject Heading:	Nutrition and metabolism

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3 1 **Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-**
4 **solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with**
5 **overweight/obesity: The SWEET Project**
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39 Abstract

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

47 **Methods and Analysis:** A work package of the SWEET project, this study consists of 5
48 double-blind randomised cross-over trials which will be carried out at 5 sites across 4
49 European countries, aiming to have n=213. Five food matrices will be tested across 3
50 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends
51 and no added sugar). Participants (body mass index (BMI) 25-35 kg/m²; aged 18-60 years)
52 will consume each formulation for 14 days. The primary endpoint is composite appetite score
53 (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour
54 postprandial incremental area under the curve during clinical investigation days on day 1 and
55 14.

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

60 **Trial registration number:** Clinicaltrials.gov: NCT04633681

62 Strengths and Limitations of this study:

- 63 • The trial is the first of its kind to investigate the effects of acute and repeated exposure to
64 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety
65 of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary
66 (chocolate) and breakfast cereal.
- 67 • This trial includes a large range of outcomes across behaviour, physiology, and health
68 from persons living in Northern, Central and Southern Europe.
- 69 • The COVID-19 pandemic resulted in changes to the design of the studies in the trial.
70 Originally, all products were to be tested across 2 sites, but the reduced time frame means
71 this is not possible for some products.

- 1
2
3 72 • Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be
4 73 reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will
5 74 be reported descriptively in these 2 studies where appropriate.
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9

10 76 **BACKGROUND AND RATIONALE**

11 77 The global increase in the prevalence of obesity and its associated diseases is driven by a
12 78 range of internal factors, involving genetic, behavioural, and metabolic determinants along
13 79 with permissive external factors from the physical, social, and public (nutritional) policy
14 80 environment (1). One of the main behavioural drivers involves a diet too rich in energy intake
15 81 relative to energy expenditure. Free sugar intake (derived from sugar added to foods and
16 82 beverages by the manufacturer or consumer) is one nutritional component that has gained
17 83 focus because of its low nutritional value (lack of vitamins, minerals or fibre) and its
18 84 potential to add to overall energy consumed, facilitating weight gain (2), and potential altered
19 85 appetite and endocrine responses to carbohydrates (sugars) relative to other macronutrients
20 86 (3).

21 87 Simply restricting free sugars from the diet without substitution may reduce diet palatability
22 88 or contribute to changes in sweet craving (4), particularly in women (5), resulting in poor
23 89 acceptance.. The replacement of free sugars with non-nutritive sweeteners and sweetness
24 90 enhancers (S&SEs) in food products is one method to reduce sugar intake while maintaining
25 91 acceptance and palatability of the diet. S&SEs have increasingly been employed over recent
26 92 years to reduce the energy and sugar content of foods; however, their impact on appetite- and
27 93 health-related outcomes is somewhat unclear (6).

28 94 The effect of S&SEs on appetite is difficult to summarise due the types of studies,
29 95 comparisons and S&SEs being used. One of the reasons for the current partial understanding
30 96 of the appetitive and metabolic effects of S&SEs in humans is that different S&SEs are
31 97 commonly assumed to have similar behavioural effects (6-8). Only recently, one 12-week
32 98 investigation of 4 distinct S&SEs reported directionally dissimilar effects of saccharin
33 99 compared to sucralose on body weight (9). A recent review comparing different S&SEs
34 100 suggests that some have the potential to enhance appetite, but these effects do not follow
35 101 through to subsequent energy intake (6). A recent meta-analysis detailed the impact of no- or
36 102 low-energy sweetened preloads compared to conventionally sweetened preloads on *ad*
37 103 *libitum* energy intake. They concluded that similar effects on energy intake were seen due to
38 104 only partial compensation being evident (although total energy intake was lower in the no- or
39 105 low-energy sweetener) (10). Furthermore, recent studies have highlighted that S&SEs may

1
2
3 106 reduce sweet food cravings and therefore reduce sugar intake (11) appetite and energy intake
4
5 107 (12). Overall, there is currently insufficient evidence to make a clear conclusion about the
6
7 108 effect of S&SEs on appetite and energy intake. Indeed, the 11 S&SEs that are currently
8
9 109 approved for use in the EU are chemically heterogeneous and absorbed, metabolised, and
10
11 110 excreted differently (13). Furthermore, most investigations of the relationship between S&SE
12
13 111 intake and health outcomes have used beverages as the vehicle (14) ; these have recently been
14
15 112 reviewed (15). Since the amount of S&SEs in the food supply is increasing in response to
16
17 113 consumer demand (16) and policy (e.g., 'sugar taxes'(17, 18); EU initiatives (19, 20)), there
18
19 114 is a pressing need to examine the appetite-related behavioural and metabolic consequences of
20
21 115 consuming S&SEs particularly in semi-solid and solid food matrices. Furthermore, it should
22
23 116 be acknowledged that differences between acute and longer-term effects of S&SEs may not
24
25 117 be the same (21) and this needs investigating further.

118

119 **AIMS AND OBJECTIVES**

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

124 The hypotheses are:

125 **H₁** Consumption of no added/reduced sugar products reformulated with S&SEs will result in
126 an altered incremental area under the curve (iAUC) appetite score, compared with the
127 sucrose-sweetened control product after repeated compared with acute consumption.

128

129 **H₂** There will be differences between the no added/reduced sugar and sucrose-sweetened
130 formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported
131 energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-
132 related (liver function and gastrointestinal (GI) side effects) outcomes.

133

134 **TRIAL DESIGN**

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

1
2
3 137 cross-over trial conducted across 5 intervention sites in 4 countries, with 3 product
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5 138 formulations (sucrose-sweetened control vs 2 individual S&SEs or S&SE blends) over 5
6
7 139 intervention product types (cake, biscuits, yoghurt, chocolate, and breakfast cereal matrices)
8
9 140 aiming for a total of 213 completers. The protocol is reported as per the SPIRIT reporting
10
11 141 guidelines (22). While this study addresses the short term impact of specific S&SEs vs added
12
13 142 sucrose on appetite, another work package in the SWEET project will examine the long term
14
15 143 (1-year) impact of a weight loss and weight maintenance intervention with or without S&SE
16
17 144 as part of a healthy diet (bmjopen-2022-061075).

145

146 ***Sample Size Determination***

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

149 Primary outcome: Power calculations showed that to detect a minimum difference of 8 mm in
150 appetite ratings on a 100 mm visual analogue scale (VAS) with 80% power, alpha 0.05, and
151 based on a published within-subject SD of 14.4 mm in VAS measures (23), an overall sample
152 of 53 completers (both sexes, same BMI group, across all centres) would be needed (24; p.30)
153 per matrix. We expect this sample size will be sufficient to detect iAUC differences in the
154 appetite response relative to control of around 8–10%, considered to be of practical relevance
155 (25).

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

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

167 **STUDY SETTING**

1
2
3 168 This trial is conducted across 5 intervention sites in 4 countries across 3 regions of Europe,
4
5 169 with each site testing a different product, whilst following the same protocol. Western
6
7 170 Europe: Leeds (University of Leeds, UK) will test biscuits; Liverpool (University of
8
9 171 Liverpool, UK) will test chocolate; Lyon (Centre de Recherche en Nutrition Humaine Rhône
10
11 172 Alpes, France) will test biscuits and cakes. Northern Europe: Copenhagen (University of
12
13 173 Copenhagen, Denmark) will test cereal. Southern Europe: Pamplona (University of Navarra,
14
15 174 Spain) will test yoghurt. University's of Leeds and Navarra are the leaders of this work
16
17 175 package, whilst University of Liverpool is the co-ordinating centre of the SWEET project in
18
19 176 its entirety.

20 177 **PATIENT AND PUBLIC INVOLVEMENT**

21
22 178 During the study, research staff discuss with participants about their experiences of the
23
24 179 clinical investigation days, examinations, participant information and written materials, etc.
25
26 180 with the aim to understand and improve participants' experiences in current and future
27
28 181 studies of this nature. This is also captured in an end of study survey.

30 182 **ELIGIBILITY CRITERIA**

31
32 183 Male and female adults aged 18-60 years, with a BMI 25-35 kg/m² are eligible. Participants
33
34 184 are required to regularly consume sugar-containing foods and willing to consume sugar and
35
36 185 sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-
37
38 186 26) (26) score <20 and a short sweet food frequency questionnaire score ≥ 3 of 11, in addition
39
40 187 to rating the control product as $\geq 40\%$ on a 100-point liking VAS during the taste test and be
41
42 188 willing to consume the product during the duration of the trial. All exclusion criteria are
43
44 189 listed in **Supplemental Material 1**.

46 191 **INTERVENTION**

47
48 192 Each trial will begin with an initial exposure to one of the 3 assigned product formulations
49
50 193 under controlled laboratory conditions (clinical investigation day, CIDs 1, 3, 5 – exposure
51
52 194 day 1), followed by repeated daily consumption of the same product at home for 12 (± 2
53
54 195 days) and a final exposure in the laboratory on day 14 (± 2 days) under identical conditions as
55
56 196 the first exposure (CIDs 2, 4, 6 – exposure day 14), resulting in all participants completing
57
58 197 the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be
59
60 198 followed by a wash out period of 14-21 days between formulations. During the at-home
199
200 199 periods, participants will consume a portion of the product at a time and place they choose

1
2
3 200 using a substitution strategy for similar energy/sweetness foods in their habitual diet. Foods
4
5 201 habitually consumed of an equivalent energy density/sweetness are identified using
6
7 202 participant's answers to a food frequency questionnaire and an energy equivalent guide, with
8
9 203 a decision making tree developed to identify the most suitable foods to substitute for each
10
11 204 intervention product. This strategy is supported by advice and agreement from the research
12
13 205 officer/dietitian. Compliance will be monitored by an intervention booklet completed daily
14
15 206 and by return of empty food packaging. All food products are provided in the same blinded
16
17 207 container/wrapping. The study duration for each participant will be a minimum of 70 days
18
19 208 (plus 7-14 days allowance for extended washout to aid scheduling of CIDs).

209

210 **Recruitment and Screening**

211 Participants will be recruited via a variety of routes e.g., study databases, webpages, social
212 media, posters, and flyers. Potential participants will be pre-screened using an online or
213 telephonic pre-screening questionnaire in accordance with the inclusion and exclusion
214 criteria. Candidates passing pre-screening will be invited to attend an information session,
215 either online or in-person, where they will be given detailed information about the study and
216 invited to participate in a Q&A session. Candidates who wish to participate in the study will
217 provide written informed consent and sign a general data protection regulation (GDPR) form
218 before being fully screened. The screening session will be performed in-person or online, and
219 will consist of anthropometric measurements (height, weight, waist and hip circumference;
220 all confirmed in-person at CID1 for participants being screened online); eligibility
221 questionnaires (EAT-26 (26) and short sweet food frequency questionnaire); baseline
222 questionnaires (A socio-demographic questionnaire, a questionnaire to assess habitual sweet
223 food consumption, including regular and S&SE sweet foods (SWITCH sweet food frequency
224 questionnaire (SWFFQ)) (27), a questionnaire to assess habitual physical activity
225 (International Physical Activity Questionnaire (IPAQ)) (28) and a consumer perspective
226 questionnaire); an eligibility taste test of the control intervention product here participants
227 rated the pleasantness of the product on a 100mm VAS after taking one bite and chewing for
228 5 seconds (a score of >40mm was required for inclusion into the study). Candidates who pass
229 the screening session will be enrolled into the study.

230

231 **Randomisation and blinding**

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

1
2
3 234 sequences for all sites will not have any study related tasks e.g., inclusion or examination of
4
5 235 participants. Each sequence will be stratified by sex (female/male) and age group (18-45
6
7 236 years/46-60 years). When feasible, a female/male ratio of minimum 60/40 was also
8
9 237 considered to reflect the target population characteristics.

10 238 Blinding of the intervention products (reformulated and control products) will be done by the
11
12 239 manufacturers. As such, blinding of the research and central laboratory staff will take place
13
14 240 allowing for a double-blind intervention. Moreover, the statistical analyses of the main
15
16 241 outcome variable will be done without breaking the intervention product-assignment code
17
18 242 before the analyses are finalised.

19 243

20 244

21
22 245 **[Figure 1]**

23 246

24 247 **Clinical investigation days**

25
26 248 Prior to each CID, participants will be asked to consume a similar evening meal at the same
27
28 249 time, before fasting for a minimum of 12 hours and a maximum of 15 hours. High intensity
29
30 250 physical activity, alcohol, and coffee will not be allowed for 12 hours before arriving to the
31
32 251 laboratory. Two glasses, approximately 500 mL, of non-carbonated water will be allowed
33
34 252 during the fasting period. Participants will provide a spot urine sample collected max 24
35
36 253 hours before each CID and will be analysed for the presence of specific S&SEs.

37 254

38
39 255 The CID procedures are outlined in Figure 2. CID start times will be scheduled in the
40
41 256 morning between 8.00 am and 10.30 am and participants will start all 6 CIDs at the same
42
43 257 time. Participants will complete a protocol compliance questionnaire to verify the above
44
45 258 requirements regarding diet, physical activity, etc. If compliance has been breached, staff will
46
47 259 reschedule the CID (within the maximum 14 days allowed, otherwise a protocol deviation
48
49 260 will be recorded). If compliance has been achieved, participants will then fill in the Control
50
51 261 of Eating Questionnaire (CoEQ)(29) to assess cravings over the last 7 days, followed by a
52
53 262 body weight measurement. Participants will consume 200 mL of water before having an
54
55 263 intravenous cannula inserted into an antecubital vein by qualified personnel. A baseline
56
57 264 fasting blood sample will be taken 15 minutes after insertion of the cannula. Once the fasting
58
59 265 sample has been taken, participants will complete fasting subjective appetite ratings for
60
266 hunger, fullness, thirst, desire to eat, prospective intake, nausea, bloating, appetite for

1
2
3 267 something savoury and for something sweet on a validated 100-point VAS accessed via a PC
4
5 268 or a tablet (30, 31). These measures will be completed on an electronic Questionnaire
6
7 269 Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be
8
9 270 measured using a culturally adapted version of the Leeds Food Preference Questionnaire
10
11 271 (LFPQ)(32) on a computer desktop. Appetite sensations measured by VAS will be repeated
12
13 272 after the LFPQ and before the researcher brings the blinded intervention product served with
14
15 273 200 mL of water. The participant will be instructed to take one bite, then answer questions
16
17 274 regarding sensory-specific satiety and expected satiety by VAS (33, 34). The participant will
18
19 275 be asked to consume the rest of the product over a period of 5-10 minutes, depending on the
20
21 276 time required to consume the matrix and asked to complete a set of appetite sensation
22
23 277 questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture
24
25 278 peak PP response (35) (yoghurt will be consumed faster than other products therefore blood
26
27 279 samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will
28
29 280 then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30,
30
31 281 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In
32
33 282 between measurements, participants will remain seated in a quiet area, free from food-related
34
35 283 sensory stimuli and read/listen to music/use a computer (provided there is no material with
36
37 284 reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete
38
39 285 the participant will be offered water or a snack before leaving the laboratory. Participants will
40
41 286 be reminded about the consumption of the products at home and that they will receive a
42
43 287 phone call the next day to complete a 24-hour diet recall and report any GI symptoms.
44
45 288 Following the end of the trial, participants will be debriefed if requested and offered the
46
47 289 chance to complete a survey about the conduct of the study.
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[Figure 2]

295 **Intervention products**

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

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

306

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

312

313 Data Collection and Outcomes

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

315

316 **Table 1:** Data Collection and Timepoints for each CID

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

317 * Timepoints for PP are earlier for yoghurt study

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

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3 321 of Eating questionnaire, GLP-1- Glucagon-like peptide 1, HDL - High density lipoprotein,
4 322 LDL - Low density lipoprotein, HbA1c - Haemoglobin A1c, GI - Gastrointestinal, CID -
5 323 Clinical Investigation Day.
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10 325 **Primary Outcome**

11 326 This trial has one primary outcome which is the iAUC for the 180-minute composite appetite
12 327 score based on hunger, fullness (reverse scored), desire to eat and prospective food
13 328 consumption (36). These subjective appetite ratings will be measured throughout the CIDs
14 329 using VAS on the QDP. The trapezoid method will be used for the calculation of iAUC (25).
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20 331 **Secondary Outcomes**

21 332 *Food preference and reward*

22 333 Food preference and food reward will be measured at all CIDs using the LFPQ (32). Changes
23 334 will be determined by comparing the relative preference/food choice, explicit liking and
24 335 implicit wanting for high-fat sweet, low-fat sweet, high-fat savoury and low-fat savoury
25 336 foods, and fat/sweet appeal bias scores in the fed and hungry states between the reformulated
26 337 and control products.
27
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31 338

32 339 *Food cravings*

33 340 Food cravings will be determined at all CIDs by craving control, craving for sweet and
34 341 savoury scores from the CoEQ (29), which is a 21-item questionnaire with responses
35 342 recorded on a 100-point VAS (1 item allows for text response).
36
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40 343

41 344 *Energy intake*

42 345 Energy intake will be measured by a 24-hour dietary recall (using the multiple pass method
43 346 (37)), which will be conducted by a trained dietitian or research staff over the telephone.

44 347 Participants will be asked to recall all food and drink consumed during the 24-hour period
45 348 since leaving the laboratory. Participants will receive training on reporting food portions
46 349 using the Australian Health Survey Food Model Booklet (38) or similar culturally adapted
47 350 resources.
48
49

50 351 Compensatory eating behaviour will be determined from the analysis of the 24-hour dietary
51 352 interview data using energy intakes calculated with national nutritional software. The following
52 353 variables will be considered: 1) Energy and macronutrient distribution and 2) Percent energy
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3 354 compensation (%EC), defined as the adjustment of energy intake (EI) provoked by the
4 intervention products (39), (see **Supplemental Material 3** for further information).
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7 356

8 357 ***Expected satiety and sensory specific satiety***

9
10 358 Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (33, 40) and
11 sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire
12 (34) after one bite and full consumption (10') of the product. Responses to both
13 questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will
14 be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).
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19 363

20 364 ***Other behavioural ratings***

21
22 365 Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will
23 be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).
24
25
26 367

27 368 ***Biochemical measures***

28
29 369 Blood for plasma analyses will be centrifuged at 1500 g at 4°C for 10 minutes immediately
30 after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before
31 being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately
32 after collection. Plasma and serum aliquots will be stored at -80°C until shipment for
33 analyses to Bioaitriki Laboratories (central lab) in Athens, Greece.
34
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36 373 Insulin concentrations will be determined by chemiluminescent microparticle immunoassay
37 (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system.
38
39 375 Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open
40 automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay
41 (Abbott) which consists of two separate concentration measurements: glycated haemoglobin
42 (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent
43 HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides
44 will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be
45 determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose
46 concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-
47 cholesterol will be determined by an accelerator selective detergent method (Ultra HDL
48 assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye
49 formation method (Direct LDL assay, Abbott). AST and ALT will be determined by
50 enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-
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3 388 carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an
4
5 389 Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated
6
7 390 according to information provided in **Supplemental Material 5**.

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9 391

10 392 *Gastrointestinal (GI) side effects*

11 393 Any reported unusual GI side effects, including abdominal pain/cramps, heartburn, stomach
12
13 394 acid/reflux, nausea, vomiting, abdominal rumbling, bloating, belching, excess gas/wind,
14
15 395 bowel movements, stool type, etc. during the study will be recorded at the phone call the day
16
17 396 after each CID and each day during the at-home intervention in a booklet including the
18
19 397 Bristol Stool Form Scale (41). The GI symptoms check has been based on the validated
20
21 398 Gastrointestinal Symptoms Rating Scale (GSRS) tool (42).

22 399

24 400 **STATISTICAL ANALYSIS PLAN**

26 401 Per protocol analysis will include participants that completed all 6 CIDs and had a level of
27
28 402 adherence to the product consumption >80%. The main evaluations for this trial will be to
29
30 403 investigate differences between the intervention products (2 no added/reduced sugar
31
32 404 reformulated S&SE products and 1 sucrose-sweetened control). Where this is not appropriate
33
34 405 for some of the secondary outcomes, descriptive analyses will be used to interpret
35
36 406 differences. Data will be pooled across the split-site (Leeds and Lyon) study using the biscuit
37
38 407 matrix. Data will be presented as means and standard deviation. Outcome variables will be
39
40 408 checked for normality and transformed where necessary. To account for any missing data,
41
42 409 analyses will be conducted using linear mixed models. Models will compare S&SE product
43
44 410 conditions vs. sucrose control in a 3 (S&SE1, S&SE2, sucrose control) x 2 (exposure day 1
45
46 411 and exposure day 14) within-subject design. Model parameters will be adjusted to obtain the
47
48 412 best model fit. Adjustments for covariates (e.g. age, gender, BMI, intervention site,
49
50 413 compliance, protocol deviations, adverse events and concomitant medication) will be applied
51
52 414 as necessary, e.g. in the event that they influence outcomes. Analyses will be reported as both
53
54 415 unadjusted and adjusted models. The American Statistical Association's policy statement on
55
56 416 p-values (43) advises that all p-values from specified statistical models be reported along
57
58 417 with point estimates, effect size and confidence intervals to help interpret the compatibility of
59
60 418 the data with the study outcomes, therefore this procedure will be followed. Otherwise, the
419
420 419 level of significance will be set at 0.05.

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3 421 ***Safety analysis***
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5 422 Information relating to adverse events (including events relating to GI side effects) and
6 423 concomitant medication will be tabulated and summarised descriptively.
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11 425 **ETHICS AND MONITORING**
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13 426 Each intervention site will obtain ethical approval from their local ethical committee. All
14 427 study procedures will be conducted in accordance with the Helsinki Declaration and the study
15 428 protocol has been registered in a public database (clinicaltrials.gov NTC04633681;
16 429 **Supplemental Material 6**). To the extent relevant and reasonable International Council for
17
18 430 Harmonisation Good Clinical Practice (ICH-GCP) guidelines will be used, and standard
19 431 operating procedures (SOP) will be developed to facilitate the same performance and
20 432 compliance with the protocol in each centre. All personal data is handled confidentially and
21 433 stored in accordance with applicable law, GDPR and local laws (see **Supplemental Material**
22 434 **7**). All participants will receive written and oral information about the study and only trained
23 435 study personnel will provide information, monitor and attest signing of the informed consent
24 436 form. Where required, monitoring of intervention sites will be performed during the study by
25 437 the University of Navarra depending on local regulations.
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35 439 **TRIAL STATUS**
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37 440 The COVID pandemic had a large impact on access to infrastructure and services across all
38 441 intervention centres. For example, research was halted in some institutions or fewer
39 442 participants could be scheduled per visit (restrictions related to distance and number of social
40 443 contacts), recruitment of new staff was frozen, new risk assessments were required, ethical
41 444 review processes were restricted or extremely prolonged because COVID-related protocols
42 445 were prioritized, procurement of supplies, consumables and services was suspended, and IT
43 446 and administrative support was restricted. Further, face-to-face clinical work was put under
44 447 strain. There were also challenges regarding staff and volunteer sickness plus overall
45 448 volunteer reluctance to engage in clinical trials affecting the speed of recruitment and testing.
46 449 Nevertheless, recruitment opened in May 2021 for the trial at the Leeds and Lyon intervention
47 450 centres using the biscuit matrix, with last participant last visit completed in June 2022 for Leeds
48 451 and expected by October 2022 for Lyon. Recruitment for the trial at Lyon using the cake matrix
49 452 opened in February 2022. The trials at Liverpool and Pamplona started recruiting in Spring
50 453 2022, and Copenhagen are still awaiting ethical approval (August 2022).
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5 **455 PUBLICATION**

6 456 After completion of the study, the findings will be submitted for publication in an
7 457 international peer-reviewed scientific open access journal and other relevant media. Research
8 458 data from the trial will be deposited in an open access online research data archive (for
9 459 example Zenodo).

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15 **461 FUNDING STATEMENT**

16 462 The present study is funded by the Horizon 2020 program: Sweeteners and sweetness
17 463 enhancers: Impact on health, obesity, safety and sustainability (acronym: SWEET, grant no:
18 464 774293). The current study was initiated by Prof. G. Finlayson as part of the Work Package 2
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20 466 Euros) to cover salary for project personnel, supplies, remuneration and dissemination of
21 467 results. The amount is deposited in a project account subject to public revision. The funder
22 468 has no role in the study design, interpretation of data or publication of material.

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31 **470 COMPETING INTERESTS STATEMENT**

32 471 JCGH, JAH and CAH and are in receipt of research funding from the American Beverage
33 472 Association. CAH has received honoraria from the International Sweeteners Association.
34 473 ARA has received honoraria from Unilever and the International Sweeteners Association.
35 474 CH's research centre provides consultancy to and has received travel funds to present
36 475 research results from organisations supported by food and drink companies. CS is a paid
37 476 employee of Cargill, Inc.

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44 **477 AUTHORS' CONTRIBUTIONS**45
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47 478

48 479 The SWEET EU-project was initiated by JH, JCGH and AR. The protocol for the present
49 480 SWEET work package intervention trial was written by CG, CHa, EA-R, SN-C, CHo, J-AN,
50 481 JAM, CS, EB, EF, HM, KB and GF, with all contributing to the design of the trial along with
51 482 BO'H, DO'C, MW, MA, MN, CR. JAM, GF, J-AN, AR and CHa are principal investigators
52 483 (PI) at the 5 intervention sites. CG and BO'H drafted the manuscript and EA-R, SN-C, MW,
53 484 CS, LK, AR, KB and GF critically reviewed the manuscript. All authors read and approved
54 485 the final manuscript. Responsible author is CG.

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486 **REFERENCES**

- 487 1. Mitchell NS, Catenacci VA, Wyatt HR, Hill JO. Obesity: overview of an epidemic.
488 *Psychiatr Clin North Am.* 2011;34(4):717-32.
- 489 2. World Health Organisation. Guideline: Sugars intake for adults and children. Geneva:
490 World Health Organisation; 2015.
- 491 3. San-Cristobal R, Navas-Carretero S, Martínez-González MÁ, Ordovas JM, Martínez
492 JA. Contribution of macronutrients to obesity: implications for precision nutrition. *Nature*
493 *Reviews Endocrinology.* 2020;16(6):305-20.
- 494 4. Anguah KO, Syed-Abdul MM, Hu Q, Jacome-Sosa M, Heimowitz C, Cox V, et al.
495 Changes in Food Cravings and Eating Behavior after a Dietary Carbohydrate Restriction
496 Intervention Trial. *Nutrients.* 2019;12(1).
- 497 5. Zellner DA, Garriga-Trillo A, Rohm E, Centeno S, Parker S. Food Liking and
498 Craving: A Cross-cultural Approach. *Appetite.* 1999;33(1):61-70.
- 499 6. O'Connor D, Pang M, Castelnuovo G, Finlayson G, Blaak E, Gibbons C, et al. A
500 rational review on the effects of sweeteners and sweetness enhancers on appetite, food
501 reward and metabolic/adiposity outcomes in adults. *Food Funct.* 2021;12(2):442-65.
- 502 7. Hunter SR, Reister EJ, Cheon E, Mattes RD. Low Calorie Sweeteners Differ in Their
503 Physiological Effects in Humans. *Nutrients.* 2019;11(11):2717.
- 504 8. Pang MD, Goossens GH, Blaak EE. The Impact of Artificial Sweeteners on Body
505 Weight Control and Glucose Homeostasis. *Frontiers in nutrition.* 2021;7:598340-.
- 506 9. Higgins KA, Mattes RD. A randomized controlled trial contrasting the effects of 4
507 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *The*
508 *American Journal of Clinical Nutrition.* 2019;109(5):1288-301.
- 509 10. Lee HY, Jack M, Poon T, Noori D, Venditti C, Hamamji S, et al. Effects of
510 Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners
511 on Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled
512 Human Intervention Studies. *Adv Nutr.* 2021;12(4):1481-99.
- 513 11. Rogers PJ, Ferriday D, Irani B, Hei Hoi JK, England CY, Bajwa KK, et al. Sweet
514 satiation: Acute effects of consumption of sweet drinks on appetite for and intake of sweet
515 and non-sweet foods. *Appetite.* 2020;149:104631.
- 516 12. Stamatakis NS, Crooks B, Ahmed A, McLaughlin JT. Effects of the Daily
517 Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A
518 Randomised Open-Label 12-Week Trial in Healthy Adults. *Nutrients.* 2020;12(10).

- 1
2
3 519 13. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate
4 520 of low-calorie sweeteners. *Nutr Rev.* 2016;74(11):670-89.
- 5 521 14. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing
6 522 metabolic derangements. *Trends Endocrinol Metab.* 2013;24(9):431-41.
- 7 523 15. McGlynn ND, Khan TA, Wang L, Zhang R, Chiavaroli L, Au-Yeung F, et al.
8 524 Association of Low- and No-Calorie Sweetened Beverages as a Replacement for Sugar-
9 525 Sweetened Beverages With Body Weight and Cardiometabolic Risk: A Systematic Review
10 526 and Meta-analysis. *JAMA Network Open.* 2022;5(3):e222092-e.
- 11 527 16. Nunn R, Young L, Ni Mhurchu C. Prevalence and Types of Non-Nutritive
12 528 Sweeteners in the New Zealand Food Supply, 2013 and 2019. *Nutrients.* 2021;13(9).
- 13 529 17. Ng SW, Colchero MA, White M. How should we evaluate sweetened beverage tax
14 530 policies? A review of worldwide experience. *BMC Public Health.* 2021;21(1):1941.
- 15 531 18. Russell C, Grimes C, Baker P, Sievert K, Lawrence MA. The drivers, trends and
16 532 dietary impacts of non-nutritive sweeteners in the food supply: a narrative review. *Nutrition*
17 533 *Research Reviews.* 2021;34(2):185-208.
- 18 534 19. Webster J. Working paper on product reformulation and portion size. 2009. Brussels:
19 535 EU Platform on diet, physical activity and health. 2016.
- 20 536 20. World Health Organisation. Sugar-sweetened beverage taxes in the WHO European
21 537 Region: success through lessons learned and challenges faced. Copenhagen. WHO Regional
22 538 Office for Europe Licence: CC BY-NC-SA 3.0 IGO; 2022. Contract No.:
23 539 WHO/EURO:2022-4781-44544-6381.
- 24 540 21. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-
25 541 energy sweetener consumption affect energy intake and body weight? A systematic review,
26 542 including meta-analyses, of the evidence from human and animal studies. *International*
27 543 *journal of obesity (2005).* 2016;40(3):381-94.
- 28 544 22. SPIRIT 2013 Statement: Defining Standard Protocol Items for Clinical Trials. *Annals*
29 545 *of Internal Medicine.* 2013;158(3):200-7.
- 30 546 23. Almiron-Roig E, Green H, Virgili R, Aeschlimann JM, Moser M, Erkner A.
31 547 Validation of a new hand-held electronic appetite rating system against the pen and paper
32 548 method. *Appetite.* 2009;53(3):465-8.
- 33 549 24. Jones BK, M. G. Design and Analysis of Cross-Over Trials. 3rd Edition ed. Boca
34 550 Raton, Florida: CRC Press; 2015.
- 35
36
37
38
39
40
41
42
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49
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53
54
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59
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2
3 551 25. Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluch A, et al. Appetite
4 552 control: Methodological aspects of the evaluation of foods. *Obesity Reviews*.
5 553 2010;11(3):251-70.
6
7
8 554 26. Garner DM, Garfinkel PE. The Eating Attitudes Test: an index of the symptoms of
9 555 anorexia nervosa. *Psychol Med*. 1979;9(2):273-9.
10
11 556 27. Masic U, Harrold JA, Christiansen P, Cuthbertson DJ, Hardman CA, Robinson E, et
12 557 al. EffectS of non-nutritive sWeetened beverages on appetITe during aCtive weighT loss
13 558 (SWITCH): Protocol for a randomized, controlled trial assessing the effects of non-nutritive
14 559 sweetened beverages compared to water during a 12-week weight loss period and a follow up
15 560 weight maintenance period. *Contemp Clin Trials*. 2017;53:80-8.
16
17 561 28. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc*
18 562 *Sport*. 2000;71 Suppl 2:114-20.
19
20 563 29. Dalton M, Finlayson G, Hill A, Blundell J. Preliminary validation and principal
21 564 components analysis of the Control of Eating Questionnaire (CoEQ) for the experience of
22 565 food craving. *European Journal of Clinical Nutrition*. 2015;69(12):1313-7.
23
24 566 30. Brunger L, Smith A, Re R, Wickham M, Philippides A, Watten P, et al. Validation of
25 567 an iPad visual analogue rating system for assessing appetite and satiety. *Appetite*.
26 568 2015;84:259-63.
27
28 569 31. Gibbons C, Caudwell P, Finlayson G, King N, Blundell J. Validation of a new hand-
29 570 held electronic data capture method for continuous monitoring of subjective appetite
30 571 sensations. *Int J Behav Nutr Phys Act*. 2011;8:57.
31
32 572 32. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit
33 573 liking and wanting for food: implications for appetite control. *Appetite*. 2008;50(1):120-7.
34
35 574 33. Forde CG, Almiron-Roig E, Brunstrom JM. Expected Satiety: Application to Weight
36 575 Management and Understanding Energy Selection in Humans. *Current obesity reports*.
37 576 2015;4(1):131-40.
38
39 577 34. Snoek HM, Huntjens L, Van Gemert LJ, De Graaf C, Weenen H. Sensory-specific
40 578 satiety in obese and normal-weight women. *Am J Clin Nutr*. 2004;80(4):823-31.
41
42 579 35. Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine Cephalic Phase
43 580 Responses to Food Cues: A Systematic Review. *Adv Nutr*. 2020;11(5):1364-83.
44
45 581 36. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association
46 582 between the effect of carbohydrates on blood glucose and subsequent short-term food intake
47 583 in young men. *The American Journal of Clinical Nutrition*. 2002;76(5):1023-30.
48
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3 584 37. Steinfeldt L, Anand J, Murayi T. Food Reporting Patterns in the USDA Automated
4 585 Multiple-Pass Method. *Procedia Food Science*. 2013;2:145-56.
5
6 586 38. Australian Bureau of Statistics. Australian Health Survey Food Model Booklet.
7 587 Commonwealth of Australia. Canberra. 2010.
8
9 588 39. Zandstra EH, Mathey MF, Graaf C, van Staveren WA. Short-term regulation of food
10 589 intake in children, young adults and the elderly. *Eur J Clin Nutr*. 2000;54(3):239-46.
11
12 590 40. Brunstrom JM, Shakeshaft NG, Scott-Samuel NE. Measuring 'expected satiety' in a
13 591 range of common foods using a method of constant stimuli. *Appetite*. 2008;51(3):604-14.
14
15 592 41. O'Donnell LJ, Virjee J, Heaton KW. Detection of pseudodiarrhoea by simple clinical
16 593 assessment of intestinal transit rate. *BMJ (Clinical research ed)*. 1990;300(6722):439-40.
17
18 594 42. Svedlund J, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal
19 595 symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci*.
20 596 1988;33(2):129-34.
21
22 597 43. Wasserstein RL, Lazar NA. The ASA Statement on p-Values: Context, Process, and
23 598 Purpose. *The American Statistician*. 2016;70(2):129-33.
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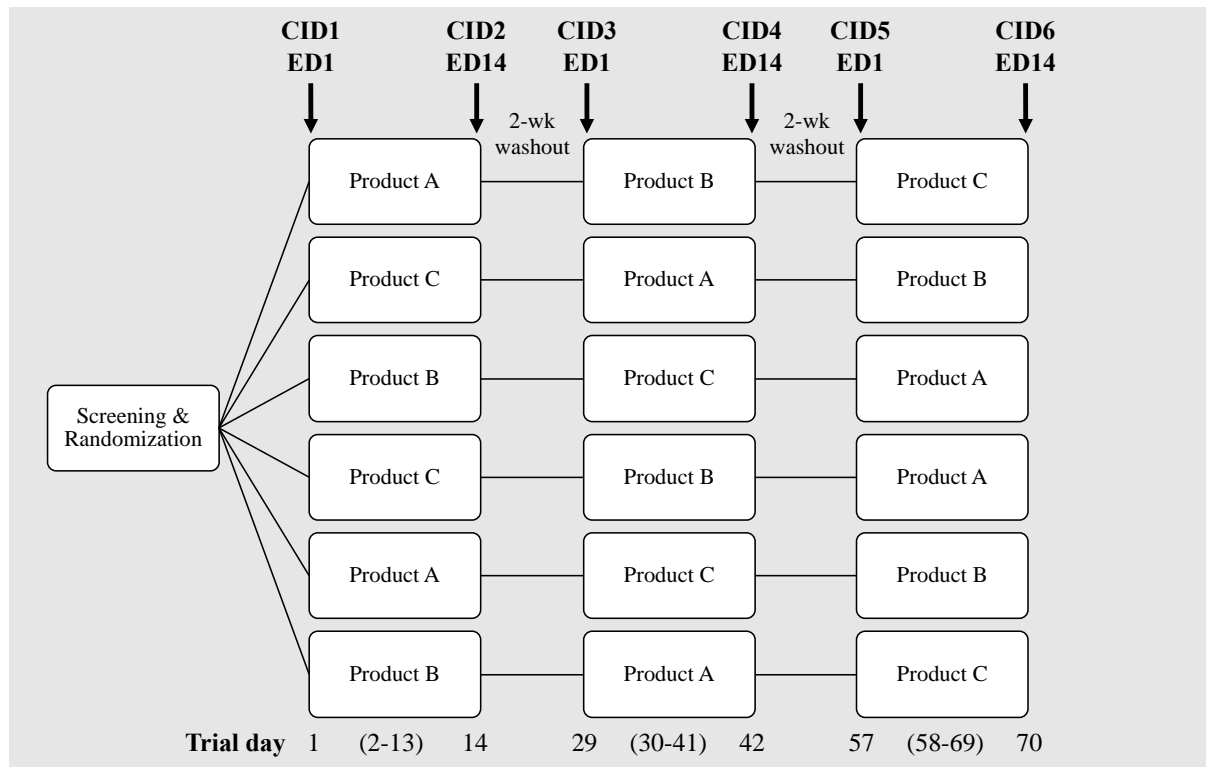


Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to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

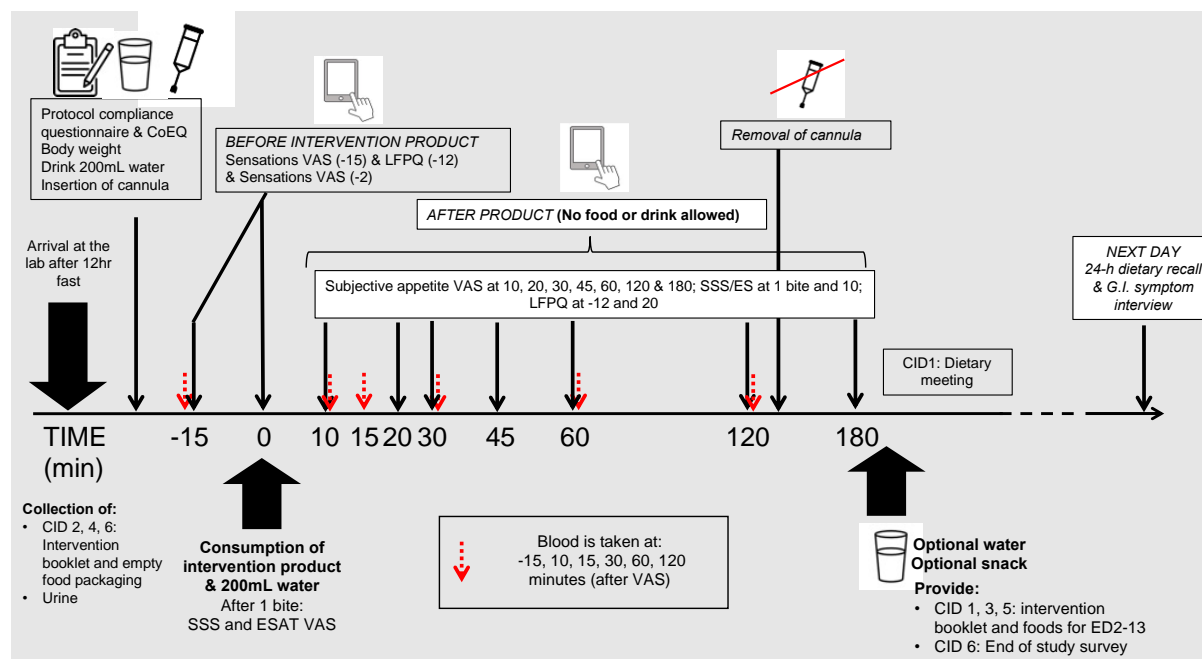


Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix.

CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.

Supplemental Material 1: Exclusion Criteria

General Criteria

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

Medical conditions as known by the person

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

Medication

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

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

- 10 • Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e.,
11 the medication is allowed if the participant has been on a stable dose for at least 3 months).
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Supplemental Material 2: Product Ingredients List

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

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

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

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Supplementary Material 3 – Energy Intake Calculation

Percent energy compensation (%EC) was derived from the dietary recall data as previously reported by Zandstra et al.¹, and Almiron-Roig et al². Briefly, %EC was calculated as:

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

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

1. Zandstra, E. H., Mathey, M. F., Graaf, C., & van Staveren, W. A. (2000). Short-term regulation of food intake in children, young adults and the elderly. *European Journal of Clinical Nutrition*, 54(3), 239-246. <http://www.ncbi.nlm.nih.gov/pubmed/10713747>
2. Almiron-Roig, E., Palla, L., Guest, K., Ricchiutu, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews*, 71(7), 458-473. [https://doi.org/10.1111.nure.12048](https://doi.org/10.1111/nure.12048)

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3 **Supplemental Material 4: Questions used to assess product taste text, subjective**
4 **appetite sensations, sensory specific satiety and expected satiety**
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8 **Food Taste Test (FTT) (Conducted at screening)**
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11 **Screen 1**
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13 You will now be presented with a food that we will ask you to evaluate. Please follow the
14 instructions as they appear on the screen.
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22 **Screen 2**
23

- 24
25 1. Take a mouthful of the food provided.
26 2. Chew while counting to 5.
27 3. Swallow.
28 4. Answer the question by moving the arrow to the left or to the right.
29
30

31 **How pleasant was this food?**
32
33 Not at all pleasant Extremely
34 pleasant
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40 -----
41 **Screen 3**
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43 Thank you. This is the end of the taste test.
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45 Please call the investigator after submitting your answer.
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Subjective Appetite Questions (used during all CIDs)

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

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

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

After 1 bite:

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

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

At 10 minutes:

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

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

After 1 bite:

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

At 10 minutes after full consumption and after SSS rating:

How much will this food stop you from feeling hungry between meals?

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

Calculation of Fatty Liver Index:

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

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

Calculation of Triglyceride Glucose Index:

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

$$\text{TyG index} = \text{Ln} [(\text{fasting triglycerides}) (\text{mg/dL}) \times \text{fasting glucose} (\text{mg/dL})] / 2$$

1. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterology*, 6(1), 33. <https://doi.org/10.1186/1471-230X-6-33>
2. Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, 6(4), 299–304. <https://doi.org/10.1089/met.2008.0034>

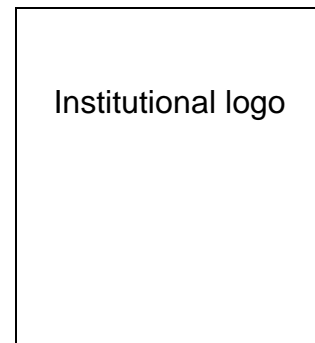
Protocol for acute and repeated dose impact of sweeteners and sweetness enhancers on appetite-related behaviour, physiology, and health: a multi-centre, double-blind, cross-over, randomised, controlled trial in people with overweight/obesity. The SWEET project.

Supplemental Material 6: Trial Registration Data Set

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

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3	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering
4	medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable
5	dose for at least 3 months)
6	Interventional
7	Allocation: Randomised. Double-blind, within-subjects, cross-over trial
8	
9	Study type
10	Primary purpose: Evaluation
11	Phase: N/A
12	Date of first enrolment
13	April 2021
14	Target sample size
15	213
16	Recruitment status
17	Recruiting
18	Primary outcome(s)
19	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
20	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of
21	Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose
22	Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers:
23	Glucagon-like peptide-1 (GLP-1) Incremental area under the curve for blood GLP-1 concentrations in response to each product
24	(120 min post intake); Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product
25	(120 min post intake).
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Appendices: Informed Consent Materials and GDPR



Informed Consent Form

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

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

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

Appendices: Informed Consent Materials and GDPR

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

Participant name: _____

Date: _____ Signature: _____

Appendices: Informed Consent Materials and GDPR

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

Consent from the study staff that provided the oral information:

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

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

Study staff name: _____

Date: _____ Signature: _____

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



Institutional logo

General Data Protection Regulation (GDPR) information for study participants

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

Appendices: Informed Consent Materials and GDPR

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

1. Which data will be collected and how?

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

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

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

2. How will my data be stored?

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

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

Appendices: Informed Consent Materials and GDPR

3. How long will my data be stored for?

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

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

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

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

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

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

Appendices: Informed Consent Materials and GDPR

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

5. How will my data be used?

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

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

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

6. Who will have access to my data?

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

Appendices: Informed Consent Materials and GDPR

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

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

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

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

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

8. How will my data be destroyed?

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

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

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

Appendices: Informed Consent Materials and GDPR

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

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

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

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

Participant's signature:

Date
Signature

Name

Researcher's signature:

Date
Signature

Name

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

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

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

1	Roles and	#5b	Name and contact information for the trial	15
2	responsibilities:		sponsor	
3	sponsor contact			
4	information			
5				
6				
7	Roles and	#5c	Role of study sponsor and funders, if any, in	15
8	responsibilities:		study design; collection, management, analysis,	
9	sponsor and funder		and interpretation of data; writing of the report;	
10			and the decision to submit the report for	
11			publication, including whether they will have	
12			ultimate authority over any of these activities	
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17	Roles and	#5d	Composition, roles, and responsibilities of the	7 and 15
18	responsibilities:		coordinating centre, steering committee,	
19	committees		endpoint adjudication committee, data	
20			management team, and other individuals or	
21			groups overseeing the trial, if applicable (see	
22			Item 21a for data monitoring committee)	
23				
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27	Introduction			
28				
29	Background and	#6a	Description of research question and justification	4
30	rationale		for undertaking the trial, including summary of	
31			relevant studies (published and unpublished)	
32			examining benefits and harms for each	
33			intervention	
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38	Background and	#6b	Explanation for choice of comparators	4
39	rationale: choice of			
40	comparators			
41				
42				
43	Objectives	#7	Specific objectives or hypotheses	5
44				
45				
46	Trial design	#8	Description of trial design including type of trial	6
47			(eg, parallel group, crossover, factorial, single	
48			group), allocation ratio, and framework (eg,	
49			superiority, equivalence, non-inferiority,	
50			exploratory)	
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**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
2				
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
9				
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14	Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
15				
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20	Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
21				
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27	Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
28				
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34	Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
35				
36				
37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
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1	Sample size	#14	Estimated number of participants needed to	6
2			achieve study objectives and how it was	
3			determined, including clinical and statistical	
4			assumptions supporting any sample size	
5			calculations	
6				
7				
8				
9	Recruitment	#15	Strategies for achieving adequate participant	8
10			enrolment to reach target sample size	
11				
12				
13	Methods:			
14	Assignment of			
15	interventions (for			
16	controlled trials)			
17				
18				
19				
20	Allocation: sequence	#16a	Method of generating the allocation sequence	8-9
21	generation		(eg, computer-generated random numbers), and	
22			list of any factors for stratification. To reduce	
23			predictability of a random sequence, details of	
24			any planned restriction (eg, blocking) should be	
25			provided in a separate document that is	
26			unavailable to those who enrol participants or	
27			assign interventions	
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33	Allocation	#16b	Mechanism of implementing the allocation	9
34	concealment		sequence (eg, central telephone; sequentially	
35	mechanism		numbered, opaque, sealed envelopes),	
36			describing any steps to conceal the sequence	
37			until interventions are assigned	
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41	Allocation:	#16c	Who will generate the allocation sequence, who	8
42	implementation		will enrol participants, and who will assign	
43			participants to interventions	
44				
45				
46	Blinding (masking)	#17a	Who will be blinded after assignment to	9
47			interventions (eg, trial participants, care	
48			providers, outcome assessors, data analysts),	
49			and how	
50				
51				
52				
53	Blinding (masking):	#17b	If blinded, circumstances under which unblinding	N/A: No safety/critical
54	emergency		is permissible, and procedure for revealing a	efficacy issues present
55	unblinding		participant's allocated intervention during the trial	
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1 **Methods: Data**
 2 **collection,**
 3 **management, and**
 4 **analysis**
 5
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7			
8	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
9			15; Supplemental Material 4
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22	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
23			N/A Data from participants who discontinue/ deviate from protocol not collected
24			
25			
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30	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
31			15; appendices
32			
33			
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40	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
41			14
42			
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47	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
48			N/A, no analysis included
49			
50			
51	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
52			N/A, no analysis included
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Methods:**Monitoring**

1			
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4			
5	Data monitoring:	#21a	Composition of data monitoring committee
6	formal committee		(DMC); summary of its role and reporting
7			structure; statement of whether it is independent
8			from the sponsor and competing interests; and
9			reference to where further details about its
10			charter can be found, if not in the protocol.
11			Alternatively, an explanation of why a DMC is
12			not needed
13			
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18	Data monitoring:	#21b	Description of any interim analyses and stopping
19	interim analysis		guidelines, including who will have access to
20			these interim results and make the final decision
21			to terminate the trial
22			
23			
24	Harms	#22	Plans for collecting, assessing, reporting, and
25			managing solicited and spontaneously reported
26			adverse events and other unintended effects of
27			trial interventions or trial conduct
28			
29			
30			
31	Auditing	#23	Frequency and procedures for auditing trial
32			conduct, if any, and whether the process will be
33			independent from investigators and the sponsor
34			
35			
36			
37	Ethics and		
38	dissemination		
39			
40	Research ethics	#24	Plans for seeking research ethics committee /
41	approval		institutional review board (REC / IRB) approval
42			
43			
44	Protocol	#25	Plans for communicating important protocol
45	amendments		modifications (eg, changes to eligibility criteria,
46			outcomes, analyses) to relevant parties (eg,
47			investigators, REC / IRBs, trial participants, trial
48			registries, journals, regulators)
49			
50			
51			
52	Consent or assent	#26a	Who will obtain informed consent or assent from
53			potential trial participants or authorised
54			surrogates, and how (see Item 32)
55			
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N/A: No safety/critical efficacy issues present

N/A: No safety/critical efficacy issues present

N/A: No safety/critical efficacy issues present

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N/A

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1	Consent or assent:	#26b	Additional consent provisions for collection and	N/A
2	ancillary studies		use of participant data and biological specimens	
3			in ancillary studies, if applicable	
4				
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6	Confidentiality	#27	How personal information about potential and	Appendices
7			enrolled participants will be collected, shared,	
8			and maintained in order to protect confidentiality	
9			before, during, and after the trial	
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13	Declaration of	#28	Financial and other competing interests for	15
14	interests		principal investigators for the overall trial and	
15			each study site	
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17				
18	Data access	#29	Statement of who will have access to the final	15
19			trial dataset, and disclosure of contractual	
20			agreements that limit such access for	
21			investigators	
22				
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25	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial	N/A: No safety/critical
26	trial care		care, and for compensation to those who suffer	efficacy issues present
27			harm from trial participation	
28				
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30				
31	Dissemination	#31a	Plans for investigators and sponsor to	15
32	policy: trial results		communicate trial results to participants,	
33			healthcare professionals, the public, and other	
34			relevant groups (eg, via publication, reporting in	
35			results databases, or other data sharing	
36			arrangements), including any publication	
37			restrictions	
38				
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41				
42	Dissemination	#31b	Authorship eligibility guidelines and any intended	16
43	policy: authorship		use of professional writers	
44				
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46	Dissemination	#31c	Plans, if any, for granting public access to the full	15
47	policy: reproducible		protocol, participant-level dataset, and statistical	
48	research		code	
49				
50				
51	Appendices			
52				
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54	Informed consent	#32	Model consent form and other related	Appendices
55	materials		documentation given to participants and	
56			authorised surrogates	
57				
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1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and 12
2 storage of biological specimens for genetic or
3 molecular analysis in the current trial and for
4 future use in ancillary studies, if applicable
5
6
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8 None The SPIRIT Explanation and Elaboration paper is distributed under the terms of the Creative
9 Commons Attribution License CC-BY-NC. This checklist can be completed online using
10 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
11 [Penelope.ai](#)
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BMJ Open

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

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Primary Subject Heading:	Nutrition and metabolism

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Secondary Subject Heading:	Public health
Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, PUBLIC HEALTH, NUTRITION & DIETETICS, General endocrinology < DIABETES & ENDOCRINOLOGY



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3 1 **Acute and repeated impact of sweeteners and sweetness enhancers in solid and semi-**
4 **solid foods on appetite - protocol for a multi-centre, cross-over, RCT in people with**
5 **overweight/obesity: The SWEET Project**
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39 Abstract

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

47 **Methods and Analysis:** A work package of the SWEET project, this study consists of 5
48 double-blind randomised cross-over trials which will be carried out at 5 sites across 4
49 European countries, aiming to have n=213. Five food matrices will be tested across 3
50 formulations (sucrose-sweetened control versus 2 reformulated products with S&SE blends
51 and no added sugar). Participants (body mass index (BMI) 25-35 kg/m²; aged 18-60 years)
52 will consume each formulation for 14 days. The primary endpoint is composite appetite score
53 (hunger, inverse of fullness, desire to eat and prospective food consumption) over a 3-hour
54 postprandial incremental area under the curve during clinical investigation days on day 1 and
55 14.

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

60 **Trial registration number:** Clinicaltrials.gov: NCT04633681

62 Strengths and Limitations of this study:

- 63 • The trial is the first of its kind to investigate the effects of acute and repeated exposure to
64 2 individual S&SE and 2 S&SE blends in 5 different sweet food products across a variety
65 of matrices including bakery (cakes and biscuits), dairy (yoghurt), confectionary
66 (chocolate) and breakfast cereal.
- 67 • This trial includes a large range of outcomes across behaviour, physiology, and health
68 from persons living in Northern, Central and Southern Europe.
- 69 • The COVID-19 pandemic resulted in changes to the design of the studies in the trial.
70 Originally, all products were to be tested across 2 sites, but the reduced time frame means
71 this is not possible for some products.

- 1
2
3 72 • Due to COVID-19 disruptions the number of participants in 2 of the 5 studies will be
4 73 reduced. Blood samples will not be taken in one of these smaller studies. Outcomes will
5 74 be reported descriptively in these 2 studies where appropriate.
6
7 75
8
9

10 76 **BACKGROUND AND RATIONALE**

11 77 The global increase in the prevalence of obesity and its associated diseases is driven by a
12 78 range of internal factors, involving genetic, behavioural, and metabolic determinants along
13 79 with permissive external factors from the physical, social, and public (nutritional) policy
14 80 environment (1). One of the main behavioural drivers involves a diet too rich in energy intake
15 81 relative to energy expenditure. Free sugar intake (derived from sugar added to foods and
16 82 beverages by the manufacturer or consumer) is one nutritional component that has gained
17 83 focus because of its low nutritional value (lack of vitamins, minerals or fibre) and its
18 84 potential to add to overall energy consumed, facilitating weight gain (2), and potential altered
19 85 appetite and endocrine responses to carbohydrates (sugars) relative to other macronutrients
20 86 (3).

21 87 Simply restricting free sugars from the diet without substitution may reduce diet palatability
22 88 or contribute to changes in sweet craving (4), particularly in women (5), resulting in poor
23 89 acceptance.. The replacement of free sugars with non-nutritive sweeteners and sweetness
24 90 enhancers (S&SEs) in food products is one method to reduce sugar intake while maintaining
25 91 acceptance and palatability of the diet. S&SEs have increasingly been employed over recent
26 92 years to reduce the energy and sugar content of foods; however, their impact on appetite- and
27 93 health-related outcomes is somewhat unclear (6).

28 94 The effect of S&SEs on appetite is difficult to summarise due the types of studies,
29 95 comparisons and S&SEs being used. One of the reasons for the current partial understanding
30 96 of the appetitive and metabolic effects of S&SEs in humans is that different S&SEs are
31 97 commonly assumed to have similar behavioural effects (6-8). Only recently, one 12-week
32 98 investigation of 4 distinct S&SEs reported directionally dissimilar effects of saccharin
33 99 compared to sucralose on body weight (9). A recent review comparing different S&SEs
34 100 suggests that some have the potential to enhance appetite, but these effects do not follow
35 101 through to subsequent energy intake (6). A recent meta-analysis detailed the impact of no- or
36 102 low-energy sweetened preloads compared to conventionally sweetened preloads on *ad*
37 103 *libitum* energy intake. They concluded that similar effects on energy intake were seen due to
38 104 only partial compensation being evident (although total energy intake was lower in the no- or
39 105 low-energy sweetener) (10). Furthermore, recent studies have highlighted that S&SEs may

1
2
3 106 reduce sweet food cravings and therefore reduce sugar intake (11) appetite and energy intake
4
5 107 (12). Overall, there is currently insufficient evidence to make a clear conclusion about the
6
7 108 effect of S&SEs on appetite and energy intake. Indeed, the 11 S&SEs that are currently
8
9 109 approved for use in the EU are chemically heterogeneous and absorbed, metabolised, and
10
11 110 excreted differently (13). Furthermore, most investigations of the relationship between S&SE
12
13 111 intake and health outcomes have used beverages as the vehicle (14) ; these have recently been
14
15 112 reviewed (15). Since the amount of S&SEs in the food supply is increasing in response to
16
17 113 consumer demand (16) and policy (e.g., 'sugar taxes'(17, 18); EU initiatives (19, 20)), there
18
19 114 is a pressing need to examine the appetite-related behavioural and metabolic consequences of
20
21 115 consuming S&SEs particularly in semi-solid and solid food matrices. Furthermore, it should
22
23 116 be acknowledged that differences between acute and longer-term effects of S&SEs may not
24
25 117 be the same (21) and this needs investigating further.

118

119 **AIMS AND OBJECTIVES**

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

124 The hypotheses are:

125 **H₁** Consumption of no added/reduced sugar products reformulated with S&SEs will result in
126 an altered incremental area under the curve (iAUC) appetite score, compared with the
127 sucrose-sweetened control product after repeated compared with acute consumption.

128

129 **H₂** There will be differences between the no added/reduced sugar and sucrose-sweetened
130 formulations on behavioural (e.g., food reward and preferences, food cravings, self-reported
131 energy intake), metabolic (satiety peptides, glycaemic and lipaemic response) and health-
132 related (liver function and gastrointestinal (GI) side effects) outcomes.

133

134 **TRIAL DESIGN**

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

1
2
3 137 cross-over trial conducted across 5 intervention sites in 4 countries, with 3 product
4
5 138 formulations (sucrose-sweetened control vs 2 individual S&SEs or S&SE blends) over 5
6
7 139 intervention product types (cake, biscuits, yoghurt, chocolate, and breakfast cereal matrices)
8
9 140 aiming for a total of 213 completers. The protocol is reported as per the SPIRIT reporting
10
11 141 guidelines (22). While this study addresses the short term impact of specific S&SEs vs added
12
13 142 sucrose on appetite, another work package in the SWEET project will examine the long term
14
15 143 (1-year) impact of a weight loss and weight maintenance intervention with or without S&SE
16
17 144 as part of a healthy diet (23).

145

146 ***Sample Size Determination***

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

149 Primary outcome: Power calculations showed that to detect a minimum difference of 8 mm in
150 appetite ratings on a 100 mm visual analogue scale (VAS) with 80% power, alpha 0.05, and
151 based on a published within-subject SD of 14.4 mm in VAS measures (24), an overall sample
152 of 53 completers (both sexes, same BMI group, across all centres) would be needed (25; p.30)
153 per matrix. We expect this sample size will be sufficient to detect iAUC differences in the
154 appetite response relative to control of around 8–10%, considered to be of practical relevance
155 (26).

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

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

167 **STUDY SETTING**

1
2
3 168 This trial is conducted across 5 intervention sites in 4 countries across 3 regions of Europe,
4
5 169 with each site testing a different product, whilst following the same protocol. Western
6
7 170 Europe: Leeds (University of Leeds, UK) will test biscuits; Liverpool (University of
8
9 171 Liverpool, UK) will test chocolate; Lyon (Centre de Recherche en Nutrition Humaine Rhône
10
11 172 Alpes, France) will test biscuits and cakes. Northern Europe: Copenhagen (University of
12
13 173 Copenhagen, Denmark) will test cereal. Southern Europe: Pamplona (University of Navarra,
14
15 174 Spain) will test yoghurt. University's of Leeds and Navarra are the leaders of this work
16
17 175 package, whilst University of Liverpool is the co-ordinating centre of the SWEET project in
18
19 176 its entirety.

20 177 **PATIENT AND PUBLIC INVOLVEMENT**

21
22 178 During the study, research staff discuss with participants about their experiences of the
23
24 179 clinical investigation days, examinations, participant information and written materials, etc.
25
26 180 with the aim to understand and improve participants' experiences in current and future
27
28 181 studies of this nature. This is also captured in an end of study survey.

30 182 **ELIGIBILITY CRITERIA**

31
32 183 Male and female adults aged 18-60 years, with a BMI 25-35 kg/m² are eligible. Participants
33
34 184 are required to regularly consume sugar-containing foods and willing to consume sugar and
35
36 185 sweetened food products. During screening, they must have an Eating Attitudes Test (EAT-
37
38 186 26) (27) score <20 and a short sweet food frequency questionnaire score ≥3 of 11, in addition
39
40 187 to rating the control product as ≥40% on a 100-point liking VAS during the taste test and be
41
42 188 willing to consume the product during the duration of the trial. All exclusion criteria are
43
44 189 listed in **Supplemental Material 1**.

46 191 **INTERVENTION**

47
48 192 Each trial will begin with an initial exposure to one of the 3 assigned product formulations
49
50 193 under controlled laboratory conditions (clinical investigation day, CIDs 1, 3, 5 – exposure
51
52 194 day 1), followed by repeated daily consumption of the same product at home for 12 (± 2
53
54 195 days) and a final exposure in the laboratory on day 14 (± 2 days) under identical conditions as
55
56 196 the first exposure (CIDs 2, 4, 6 – exposure day 14), resulting in all participants completing
57
58 197 the 3 products formulations in a Latin square design (see Figure 1). CIDs 2 and 4 will be
59
60 198 followed by a wash out period of 14-21 days between formulations. During the at-home
199
200 199 periods, participants will consume a portion of the product at a time and place they choose

1
2
3 200 using a substitution strategy for similar energy/sweetness foods in their habitual diet. Foods
4
5 201 habitually consumed of an equivalent energy density/sweetness are identified using
6
7 202 participant's answers to a food frequency questionnaire and an energy equivalent guide, with
8
9 203 a decision making tree developed to identify the most suitable foods to substitute for each
10
11 204 intervention product. This strategy is supported by advice and agreement from the research
12
13 205 officer/dietitian. Compliance will be monitored by an intervention booklet completed daily
14
15 206 and by return of empty food packaging. All food products are provided in the same blinded
16
17 207 container/wrapping. The study duration for each participant will be a minimum of 70 days
18
19 208 (plus 7-14 days allowance for extended washout to aid scheduling of CIDs).

209

210 **Recruitment and Screening**

211 Participants will be recruited via a variety of routes e.g., study databases, webpages, social
212 media, posters, and flyers. Potential participants will be pre-screened using an online or
213 telephonic pre-screening questionnaire in accordance with the inclusion and exclusion
214 criteria. Candidates passing pre-screening will be invited to attend an information session,
215 either online or in-person, where they will be given detailed information about the study and
216 invited to participate in a Q&A session. Candidates who wish to participate in the study will
217 provide written informed consent and sign a general data protection regulation (GDPR) form
218 before being fully screened. The screening session will be performed in-person or online, and
219 will consist of anthropometric measurements (height, weight, waist and hip circumference;
220 all confirmed in-person at CID1 for participants being screened online); eligibility
221 questionnaires (EAT-26 (27) and short sweet food frequency questionnaire); baseline
222 questionnaires (A socio-demographic questionnaire, a questionnaire to assess habitual sweet
223 food consumption, including regular and S&SE sweet foods (SWITCH sweet food frequency
224 questionnaire (SWFFQ)) (28), a questionnaire to assess habitual physical activity
225 (International Physical Activity Questionnaire (IPAQ)) (29) and a consumer perspective
226 questionnaire); an eligibility taste test of the control intervention product here participants
227 rated the pleasantness of the product on a 100mm VAS after taking one bite and chewing for
228 5 seconds (a score of >40mm was required for inclusion into the study). Candidates who pass
229 the screening session will be enrolled into the study.

230

231 **Randomisation and blinding**

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

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

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

243

244

245 **[Figure 1]**

246

247 **Clinical investigation days**

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

254

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

1
2
3 267 something savoury and for something sweet on a validated 100-point VAS accessed via a PC
4
5 268 or a tablet (31, 32). These measures will be completed on an electronic Questionnaire
6
7 269 Delivery Platform (QDP), using separate screens for each VAS. Next, food reward will be
8
9 270 measured using a culturally adapted version of the Leeds Food Preference Questionnaire
10
11 271 (LFPQ)(33) on a computer desktop. Appetite sensations measured by VAS will be repeated
12
13 272 after the LFPQ and before the researcher brings the blinded intervention product served with
14
15 273 200 mL of water. The participant will be instructed to take one bite, then answer questions
16
17 274 regarding sensory-specific satiety and expected satiety by VAS (34, 35). The participant will
18
19 275 be asked to consume the rest of the product over a period of 5-10 minutes, depending on the
20
21 276 time required to consume the matrix and asked to complete a set of appetite sensation
22
23 277 questions by VAS at 10 min, followed by blood samples at 7-10 and 12-15 min to capture
24
25 278 peak PP response (36) (yoghurt will be consumed faster than other products therefore blood
26
27 279 samples will be taken earlier for this matrix). VAS for assessment of appetite sensation will
28
29 280 then be taken at 20, 30, 45, 60, 120 and 180 min with blood samples taken after VAS at 30,
30
31 281 60 and 120 min. The LFPQ will be repeated in the fed state after the 20-min VAS. In
32
33 282 between measurements, participants will remain seated in a quiet area, free from food-related
34
35 283 sensory stimuli and read/listen to music/use a computer (provided there is no material with
36
37 284 reference to food/drink). Once the 180-min appetite sensation questions by VAS is complete
38
39 285 the participant will be offered water or a snack before leaving the laboratory. Participants will
40
41 286 be reminded about the consumption of the products at home and that they will receive a
42
43 287 phone call the next day to complete a 24-hour diet recall and report any GI symptoms.
44
45 288 Following the end of the trial, participants will be debriefed if requested and offered the
46
47 289 chance to complete a survey about the conduct of the study.
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[Figure 2]

295 **Intervention products**

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

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

306

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

312

313 Data Collection and Outcomes

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

315

316 **Table 1:** Data Collection and Timepoints for each CID

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

317 * Timepoints for PP are earlier for yoghurt study

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

1
2
3 321 of Eating questionnaire, GLP-1- Glucagon-like peptide 1, HDL - High density lipoprotein,
4 322 LDL - Low density lipoprotein, HbA1c - Haemoglobin A1c, GI - Gastrointestinal, CID -
5
6 323 Clinical Investigation Day.
7

8 324
9

10 325 **Primary Outcome**

11 326 This trial has one primary outcome which is the iAUC for the 180-minute composite appetite
12 327 score based on hunger, fullness (reverse scored), desire to eat and prospective food
13
14 328 consumption (37). These subjective appetite ratings will be measured throughout the CIDs
15 329 using VAS on the QDP. The trapezoid method will be used for the calculation of iAUC (26).
16
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18

19 330

20 331 **Secondary Outcomes**

21 332 ***Food preference and reward***

22 333 Food preference and food reward will be measured at all CIDs using the LFPQ (33). Changes
23
24 334 will be determined by comparing the relative preference/food choice, explicit liking and
25
26 335 implicit wanting for high-fat sweet, low-fat sweet, high-fat savoury and low-fat savoury
27
28 336 foods, and fat/sweet appeal bias scores in the fed and hungry states between the reformulated
29
30 337 and control products.
31

32 338

33 339 ***Food cravings***

34 340 Food cravings will be determined at all CIDs by craving control, craving for sweet and
35
36 341 savoury scores from the CoEQ (30), which is a 21-item questionnaire with responses
37
38 342 recorded on a 100-point VAS (1 item allows for text response).
39
40 343

41 344

42 345 ***Energy intake***

43 346 Energy intake will be measured by a 24-hour dietary recall (using the multiple pass method
44
45 347 (38)), which will be conducted by a trained dietitian or research staff over the telephone.

46
47 348 Participants will be asked to recall all food and drink consumed during the 24-hour period
48
49 349 since leaving the laboratory. Participants will receive training on reporting food portions
50
51 350 using the Australian Health Survey Food Model Booklet (39) or similar culturally adapted
52
53 351 resources.

54
55 352 Compensatory eating behaviour will be determined from the analysis of the 24-hour dietary
56
57 353 interview data using energy intakes calculated with national nutritional software. The following
58
59 354 variables will be considered: 1) Energy and macronutrient distribution and 2) Percent energy
60

1
2
3 354 compensation (%EC), defined as the adjustment of energy intake (EI) provoked by the
4 intervention products (40), (see **Supplemental Material 3** for further information).
5
6
7 356

8 357 ***Expected satiety and sensory specific satiety***

9
10 358 Expected satiety will be measured by the Expected Satiety (ESAT) questionnaire (34, 41) and
11 sensory specific satiety will be measured by the Sensory-Specific Satiety (SSS) questionnaire
12 (35) after one bite and full consumption (10') of the product. Responses to both
13
14 360 questionnaires are recorded on a 100-point VAS completed on the QDP. ESAT and SSS will
15 361 be recorded on all CIDs (see **Supplemental Material 4** for details of each VAS).
16
17 362
18
19 363

20 364 ***Other behavioural ratings***

21
22 365 Subjective ratings of thirst, nausea, bloating, appetite for sweet and appetite for savoury will
23 be recorded using 100-point VAS on the QDP regularly throughout the CIDs (Table 1).
24
25 366
26 367

27 368 ***Biochemical measures***

28
29 369 Blood for plasma analyses will be centrifuged at 1500 g at 4°C for 10 minutes immediately
30 after being collected. Blood for serum analyses will be left to clot for 30-60 minutes before
31 being centrifuged. Whole blood samples for DNA and HbA1c will be frozen immediately
32 after collection. Plasma and serum aliquots will be stored at -80°C until shipment for
33 analyses to Bioatriki Laboratories (central lab) in Athens, Greece.
34
35 373 Insulin concentrations will be determined by chemiluminescent microparticle immunoassay
36 (CMIA) (Abbott Laboratories) using an Abbott Alinity i automated immunoassay system.
37
38 374 Ghrelin, GLP-1 and PP concentrations will be determined by ELISA, using an open
39 automated ELISA system. Haemoglobin A1c will be determined by enzymatic assay
40 (Abbott) which consists of two separate concentration measurements: glycated haemoglobin
41 (HbA1c) and total haemoglobin. The two concentrations are used to determine the percent
42 HbA1c (NGSP units) or the haemoglobin fraction in mmol/mol (IFCC units). Triglycerides
43 will be determined by glycerol phosphate oxidase method (Abbott). Total cholesterol will be
44 determined by enzymatic (oxidase, esterase and peroxidase) analysis (Abbott). Glucose
45 concentrations will be determined by enzymatic (Hexokinase/G-6-PDH) (Abbott). HDL-
46 cholesterol will be determined by an accelerator selective detergent method (Ultra HDL
47 assay, Abbott) and LDL-cholesterol by a selective resolution of LDL-Particles under dye
48 formation method (Direct LDL assay, Abbott). AST and ALT will be determined by
49 enzymatic (NADH (without P-5'-P)) assays and GGT by enzymatic, L-Gamma glutamyl-3-
50
51 387

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2
3 388 carboxy-4-nitroanilide substrate (Abbott). All biochemistry parameters will be analysed by an
4
5 389 Abbott Alinity c analyser. Fatty liver index and triglyceride glucose index will be calculated
6
7 390 according to information provided in **Supplemental Material 5**.

8
9 391

10 392 *Gastrointestinal (GI) side effects*

11 393 Any reported unusual GI side effects, including abdominal pain/cramps, heartburn, stomach
12
13 394 acid/reflux, nausea, vomiting, abdominal rumbling, bloating, belching, excess gas/wind,
14
15 395 bowel movements, stool type, etc. during the study will be recorded at the phone call the day
16
17 396 after each CID and each day during the at-home intervention in a booklet including the
18
19 397 Bristol Stool Form Scale (42). The GI symptoms check has been based on the validated
20
21 398 Gastrointestinal Symptoms Rating Scale (GSRS) tool (43).

22
23 399

24 400 **STATISTICAL ANALYSIS PLAN**

25
26 401 Per protocol analysis will include participants that completed all 6 CIDs and had a level of
27
28 402 adherence to the product consumption >80%. The main evaluations for this trial will be to
29
30 403 investigate differences between the intervention products (2 no added/reduced sugar
31
32 404 reformulated S&SE products and 1 sucrose-sweetened control). Where this is not appropriate
33
34 405 for some of the secondary outcomes, descriptive analyses will be used to interpret
35
36 406 differences. Data will be pooled across the split-site (Leeds and Lyon) study using the biscuit
37
38 407 matrix. Data will be presented as means and standard deviation. Outcome variables will be
39
40 408 checked for normality and transformed where necessary. To account for any missing data,
41
42 409 analyses will be conducted using linear mixed models. Models will compare S&SE product
43
44 410 conditions vs. sucrose control in a 3 (S&SE1, S&SE2, sucrose control) x 2 (exposure day 1
45
46 411 and exposure day 14) within-subject design. Model parameters will be adjusted to obtain the
47
48 412 best model fit. Adjustments for covariates (e.g. age, gender, BMI, intervention site,
49
50 413 compliance, protocol deviations, adverse events and concomitant medication) will be applied
51
52 414 as necessary, e.g. in the event that they influence outcomes. Analyses will be reported as both
53
54 415 unadjusted and adjusted models. The American Statistical Association's policy statement on
55
56 416 p-values (44) advises that all p-values from specified statistical models be reported along
57
58 417 with point estimates, effect size and confidence intervals to help interpret the compatibility of
59
60 418 the data with the study outcomes, therefore this procedure will be followed. Otherwise, the
419
420 419 level of significance will be set at 0.05.

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3 421 ***Safety analysis***
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5 422 Information relating to adverse events (including events relating to GI side effects) and
6 423 concomitant medication will be tabulated and summarised descriptively.
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11 425 **ETHICS AND MONITORING**
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13 426 Each intervention site has obtained ethical approval from their local ethical committee. The
14 427 following details the specific ethical committees and the reference numbers. University of
15 428 Leeds School of Psychology (PSC-127, approved 19th November 2020), Comité de
16 429 Protection des Personnes Nord-Ouest III (2021-42, approved 28th March 2022), Comité de
17 430 Ética de la Investigación de la Universidad de Navarra (2021.205, approved 7th March 2022),
18 431 The Ethical Committee, Region H. Denmark (application number H-21078447, submitted
19 432 and have made comments back to review committee, final decision is imminent) and
20 433 University of Liverpool Central University Research Ethics Committee D (10659, approved
21 434 14th April 2022). All study procedures will be conducted in accordance with the Helsinki
22 435 Declaration and the study protocol has been registered in a public database (clinicaltrials.gov
23 436 NTC04633681; **Supplemental Material 6**). To the extent relevant and reasonable
24 437 International Council for Harmonisation Good Clinical Practice (ICH-GCP) guidelines will
25 438 be used, and standard operating procedures (SOP) will be developed to facilitate the same
26 439 performance and compliance with the protocol in each centre. All personal data is handled
27 440 confidentially and stored in accordance with applicable law, GDPR and local laws (see
28 441 **Supplemental Material 7**). All participants will receive written and oral information about
29 442 the study and only trained study personnel will provide information, monitor and attest
30 443 signing of the informed consent form. Where required, monitoring of intervention sites will
31 444 be performed during the study by the University of Navarra depending on local regulations.
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47 446 **TRIAL STATUS**
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49 447 The COVID pandemic had a large impact on access to infrastructure and services across all
50 448 intervention centres. For example, research was halted in some institutions or fewer
51 449 participants could be scheduled per visit (restrictions related to distance and number of social
52 450 contacts), recruitment of new staff was frozen, new risk assessments were required, ethical
53 451 review processes were restricted or extremely prolonged because COVID-related protocols
54 452 were prioritized, procurement of supplies, consumables and services was suspended, and IT
55 453 and administrative support was restricted. Further, face-to-face clinical work was put under
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3 454 strain. There were also challenges regarding staff and volunteer sickness plus overall
4
5 455 volunteer reluctance to engage in clinical trials affecting the speed of recruitment and testing.
6
7 456 Nevertheless, recruitment opened in May 2021 for the trial at the Leeds and Lyon intervention
8
9 457 centres using the biscuit matrix, with last participant last visit completed in June 2022 for Leeds
10
11 458 and expected by October 2022 for Lyon. Recruitment for the trial at Lyon using the cake matrix
12
13 459 opened in February 2022. The trials at Liverpool and Pamplona started recruiting in Spring
14
15 460 2022, and Copenhagen are still awaiting ethical approval (August 2022).
16

461

462 **PUBLICATION**

17
18
19 463 After completion of the study, the findings will be submitted for publication in an
20
21 464 international peer-reviewed scientific open access journal and other relevant media. Research
22
23 465 data from the trial will be deposited in an open access online research data archive (for
24
25 466 example Zenodo).

467

468 **FUNDING STATEMENT**

26
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28
29 469 The present study is funded by the Horizon 2020 program: Sweeteners and sweetness
30
31 470 enhancers: Impact on health, obesity, safety and sustainability (acronym: SWEET, grant no:
32
33 471 774293). The current study was initiated by Prof. G. Finlayson as part of the Work Package 2
34
35 472 of the SWEET project. The study receives funding from the Horizon2020 program (9 million
36
37 473 Euros) to cover salary for project personnel, supplies, remuneration and dissemination of
38
39 474 results. The amount is deposited in a project account subject to public revision. The funder
40
41 475 has no role in the study design, interpretation of data or publication of material.

476

477 **COMPETING INTERESTS STATEMENT**

42
43
44 478 JCGH, JAH and CAH and are in receipt of research funding from the American Beverage
45
46 479 Association. CAH has received honoraria from the International Sweeteners Association.
47
48 480 ARA has received honoraria from Unilever and the International Sweeteners Association.
49
50 481 CH's research centre provides consultancy to and has received travel funds to present
51
52 482 research results from organisations supported by food and drink companies. CS is a paid
53
54 483 employee of Cargill, Inc.

55 56 484 **AUTHORS' CONTRIBUTIONS**

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59 485
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2
3 486 The SWEET EU-project was initiated by JH, JCGH and AR. The protocol for the present
4
5 487 SWEET work package intervention trial was written by CG, CHa, EA-R, SN-C, CHo, J-AN,
6
7 488 JAM, CS, EB, EF, HM, KB and GF, with all contributing to the design of the trial along with
8
9 489 BO'H, DO'C, MW, MA, MN, CR. JAM, GF, J-AN, AR and CHa are principal investigators
10
11 490 (PI) at the 5 intervention sites. CG and BO'H drafted the manuscript and EA-R, SN-C, MW,
12
13 491 CS, LK, AR, KB and GF critically reviewed the manuscript. All authors read and approved
14
15 492 the final manuscript. Responsible author is CG.

493 REFERENCES

- 17 494 1. Mitchell NS, Catenacci VA, Wyatt HR, Hill JO. Obesity: overview of an epidemic.
18
19 495 *Psychiatr Clin North Am.* 2011;34(4):717-32.
- 20
21 496 2. Organisation WH. Guideline: Sugars intake for adults and children. Geneva: World
22
23 497 Health Organisation; 2015.
- 24 498 3. San-Cristobal R, Navas-Carretero S, Martínez-González MÁ, Ordovas JM, Martínez
25
26 499 JA. Contribution of macronutrients to obesity: implications for precision nutrition. *Nature*
27
28 500 *Reviews Endocrinology.* 2020;16(6):305-20.
- 29 501 4. Anguah KO, Syed-Abdul MM, Hu Q, Jacome-Sosa M, Heimowitz C, Cox V, et al.
30
31 502 Changes in Food Cravings and Eating Behavior after a Dietary Carbohydrate Restriction
32
33 503 Intervention Trial. *Nutrients.* 2019;12(1).
- 34 504 5. Zellner DA, Garriga-Trillo A, Rohm E, Centeno S, Parker S. Food Liking and
35
36 505 Craving: A Cross-cultural Approach. *Appetite.* 1999;33(1):61-70.
- 37
38 506 6. O'Connor D, Pang M, Castelnuovo G, Finlayson G, Blaak E, Gibbons C, et al. A
39
40 507 rational review on the effects of sweeteners and sweetness enhancers on appetite, food
41
42 508 reward and metabolic/adiposity outcomes in adults. *Food Funct.* 2021;12(2):442-65.
- 43 509 7. Hunter SR, Reister EJ, Cheon E, Mattes RD. Low Calorie Sweeteners Differ in Their
44
45 510 Physiological Effects in Humans. *Nutrients.* 2019;11(11):2717.
- 46 511 8. Pang MD, Goossens GH, Blaak EE. The Impact of Artificial Sweeteners on Body
47
48 512 Weight Control and Glucose Homeostasis. *Frontiers in nutrition.* 2021;7:598340-.
- 49
50 513 9. Higgins KA, Mattes RD. A randomized controlled trial contrasting the effects of 4
51
52 514 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *The*
53
54 515 *American Journal of Clinical Nutrition.* 2019;109(5):1288-301.
- 55 516 10. Lee HY, Jack M, Poon T, Noori D, Venditti C, Hamamji S, et al. Effects of
56
57 517 Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners
58
59 518 on Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled
60
61 519 Human Intervention Studies. *Adv Nutr.* 2021;12(4):1481-99.

- 1
2
3 520 11. Rogers PJ, Ferriday D, Irani B, Hei Hoi JK, England CY, Bajwa KK, et al. Sweet
4 521 satiation: Acute effects of consumption of sweet drinks on appetite for and intake of sweet
5 522 and non-sweet foods. *Appetite*. 2020;149:104631.
- 6
7
8 523 12. Stamatakis NS, Crooks B, Ahmed A, McLaughlin JT. Effects of the Daily
9 524 Consumption of Stevia on Glucose Homeostasis, Body Weight, and Energy Intake: A
10 525 Randomised Open-Label 12-Week Trial in Healthy Adults. *Nutrients*. 2020;12(10).
- 11
12
13 526 13. Magnuson BA, Carakostas MC, Moore NH, Poulos SP, Renwick AG. Biological fate
14 527 of low-calorie sweeteners. *Nutr Rev*. 2016;74(11):670-89.
- 15
16
17 528 14. Swithers SE. Artificial sweeteners produce the counterintuitive effect of inducing
18 529 metabolic derangements. *Trends Endocrinol Metab*. 2013;24(9):431-41.
- 19
20
21 530 15. McGlynn ND, Khan TA, Wang L, Zhang R, Chiavaroli L, Au-Yeung F, et al.
22 531 Association of Low- and No-Calorie Sweetened Beverages as a Replacement for Sugar-
23 532 Sweetened Beverages With Body Weight and Cardiometabolic Risk: A Systematic Review
24 533 and Meta-analysis. *JAMA Network Open*. 2022;5(3):e222092-e.
- 25
26
27 534 16. Nunn R, Young L, Ni Mhurchu C. Prevalence and Types of Non-Nutritive
28 535 Sweeteners in the New Zealand Food Supply, 2013 and 2019. *Nutrients*. 2021;13(9).
- 29
30
31 536 17. Ng SW, Colchero MA, White M. How should we evaluate sweetened beverage tax
32 537 policies? A review of worldwide experience. *BMC Public Health*. 2021;21(1):1941.
- 33
34
35 538 18. Russell C, Grimes C, Baker P, Sievert K, Lawrence MA. The drivers, trends and
36 539 dietary impacts of non-nutritive sweeteners in the food supply: a narrative review. *Nutrition*
37 540 *Research Reviews*. 2021;34(2):185-208.
- 38
39
40 541 19. Webster J. Working paper on product reformulation and portion size. Brussels: EU
41 542 Platform on diet, physical activity and health
42 543 ; 2009.
- 43
44
45 544 20. Organisation WH. Sugar-sweetened beverage taxes in the WHO European Region:
46 545 success through lessons learned and challenges faced. Copenhagen. WHO Regional Office
47 546 for Europe Licence: CC BY-NC-SA 3.0 IGO; 2022. Contract No.: WHO/EURO:2022-4781-
48 547 44544-6381.
- 49
50
51 548 21. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR, et al. Does low-
52 549 energy sweetener consumption affect energy intake and body weight? A systematic review,
53 550 including meta-analyses, of the evidence from human and animal studies. *International*
54 551 *journal of obesity (2005)*. 2016;40(3):381-94.
- 55
56
57
58 552 22. SPIRIT 2013 Statement: Defining Standard Protocol Items for Clinical Trials. *Annals*
59 553 *of Internal Medicine*. 2013;158(3):200-7.

- 1
2
3 554 23. Kjølbæk LM, Y; Blaak, E; Martínez, J; Feskens, E; Finlayson, G; Andersen, S;
4 555 Reppas, K; Navas-Carretero, S; Adam, T; Hodgkins, C; del Álamo, M; Moshoyiannis, H;
5 556 Halford, J; Harrold, J; Raben, A. Protocol for a multicentre, parallel, randomised, controlled,
6 557 trial on the effect of sweeteners and sweetness enhancers on health, obesity and safety in
7 558 overweight adults and children. The SWEET project. *BMJ Open*. 2022; In preparation.
- 8 559 24. Almiron-Roig E, Green H, Virgili R, Aeschlimann JM, Moser M, Erkner A.
9 560 Validation of a new hand-held electronic appetite rating system against the pen and paper
10 561 method. *Appetite*. 2009;53(3):465-8.
- 11 562 25. Jones BK, M. G. Design and Analysis of Cross-Over Trials. 3rd Edition ed. Boca
12 563 Raton, Florida: CRC Press; 2015.
- 13 564 26. Blundell J, De Graaf C, Hulshof T, Jebb S, Livingstone B, Lluich A, et al. Appetite
14 565 control: Methodological aspects of the evaluation of foods. *Obesity Reviews*.
15 566 2010;11(3):251-70.
- 16 567 27. Garner DM, Garfinkel PE. The Eating Attitudes Test: an index of the symptoms of
17 568 anorexia nervosa. *Psychol Med*. 1979;9(2):273-9.
- 18 569 28. Masic U, Harrold JA, Christiansen P, Cuthbertson DJ, Hardman CA, Robinson E, et
19 570 al. EffectS of non-nutritive sWeetened beverages on appetITe during aCtive weigHt loss
20 571 (SWITCH): Protocol for a randomized, controlled trial assessing the effects of non-nutritive
21 572 sweetened beverages compared to water during a 12-week weight loss period and a follow up
22 573 weight maintenance period. *Contemp Clin Trials*. 2017;53:80-8.
- 23 574 29. Booth M. Assessment of physical activity: an international perspective. *Res Q Exerc*
24 575 *Sport*. 2000;71 Suppl 2:114-20.
- 25 576 30. Dalton M, Finlayson G, Hill A, Blundell J. Preliminary validation and principal
26 577 components analysis of the Control of Eating Questionnaire (CoEQ) for the experience of
27 578 food craving. *European Journal of Clinical Nutrition*. 2015;69(12):1313-7.
- 28 579 31. Brunger L, Smith A, Re R, Wickham M, Philippides A, Watten P, et al. Validation of
29 580 an iPad visual analogue rating system for assessing appetite and satiety. *Appetite*.
30 581 2015;84:259-63.
- 31 582 32. Gibbons C, Caudwell P, Finlayson G, King N, Blundell J. Validation of a new hand-
32 583 held electronic data capture method for continuous monitoring of subjective appetite
33 584 sensations. *Int J Behav Nutr Phys Act*. 2011;8:57.
- 34 585 33. Finlayson G, King N, Blundell J. The role of implicit wanting in relation to explicit
35 586 liking and wanting for food: implications for appetite control. *Appetite*. 2008;50(1):120-7.

- 1
2
3 587 34. Forde CG, Almiron-Roig E, Brunstrom JM. Expected Satiety: Application to Weight
4 588 Management and Understanding Energy Selection in Humans. *Current obesity reports*.
5 589 2015;4(1):131-40.
6
7
8 590 35. Snoek HM, Huntjens L, Van Gemert LJ, De Graaf C, Weenen H. Sensory-specific
9 591 satiety in obese and normal-weight women. *Am J Clin Nutr*. 2004;80(4):823-31.
10
11 592 36. Lasschuijt MP, Mars M, de Graaf C, Smeets PAM. Endocrine Cephalic Phase
12 593 Responses to Food Cues: A Systematic Review. *Adv Nutr*. 2020;11(5):1364-83.
13
14 594 37. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association
15 595 between the effect of carbohydrates on blood glucose and subsequent short-term food intake
16 596 in young men. *The American Journal of Clinical Nutrition*. 2002;76(5):1023-30.
17
18 597 38. Steinfeldt L, Anand J, Murayi T. Food Reporting Patterns in the USDA Automated
19 598 Multiple-Pass Method. *Procedia Food Science*. 2013;2:145-56.
20
21 599 39. Australian Health Survey. Food Model Booklet. In: Statistics ABo, editor. Belconnen
22 600 ACT2010.
23
24 601 40. Zandstra EH, Mathey MF, Graaf C, van Staveren WA. Short-term regulation of food
25 602 intake in children, young adults and the elderly. *Eur J Clin Nutr*. 2000;54(3):239-46.
26
27 603 41. Brunstrom JM, Shakeshaft NG, Scott-Samuel NE. Measuring 'expected satiety' in a
28 604 range of common foods using a method of constant stimuli. *Appetite*. 2008;51(3):604-14.
29
30 605 42. O'Donnell LJ, Virjee J, Heaton KW. Detection of pseudodiarrhoea by simple clinical
31 606 assessment of intestinal transit rate. *BMJ (Clinical research ed)*. 1990;300(6722):439-40.
32
33 607 43. Svedlund J, Sjödin I, Dotevall G. GSRS--a clinical rating scale for gastrointestinal
34 608 symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Dig Dis Sci*.
35 609 1988;33(2):129-34.
36
37 610 44. Wasserstein RL, Lazar NA. The ASA Statement on p-Values: Context, Process, and
38 611 Purpose. *The American Statistician*. 2016;70(2):129-33.
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613 **FIGURE LEGENDS**

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

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3 621 lab and repeats the study block with product B, followed by another 2-week washout,
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5 622 followed by the final study block with product C.
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8 624 **Figure 2:** Example timeline of events during Clinical Investigation Day for biscuit matrix.
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10 625 CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I.,
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12 626 gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety;
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14 627 VAS, Visual Analogue Scale.
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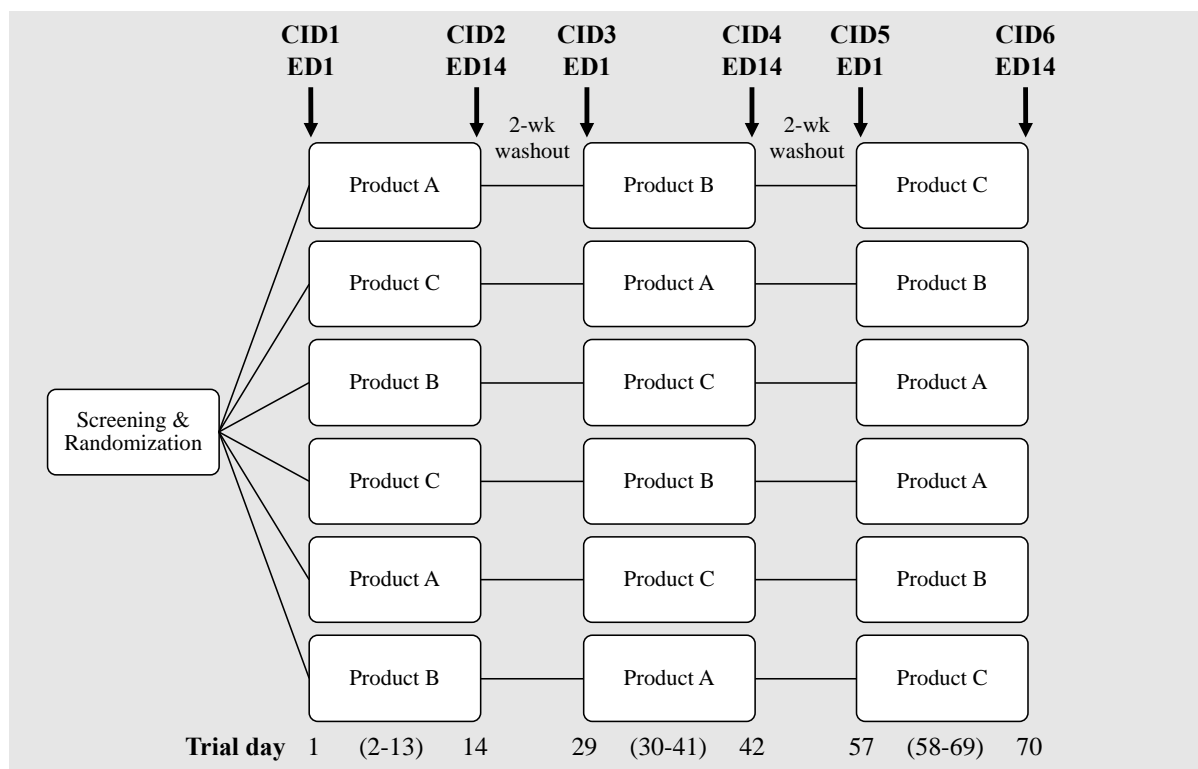


Figure 1: Latin Square design and duration for cross-over trials. Each trial will include two no added/reduced sugar reformulated products and 1 sucrose-sweetened control (double-blind) per food matrix. Participant will be randomised to 1 of 6 treatment orders. For example, a participant randomised to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

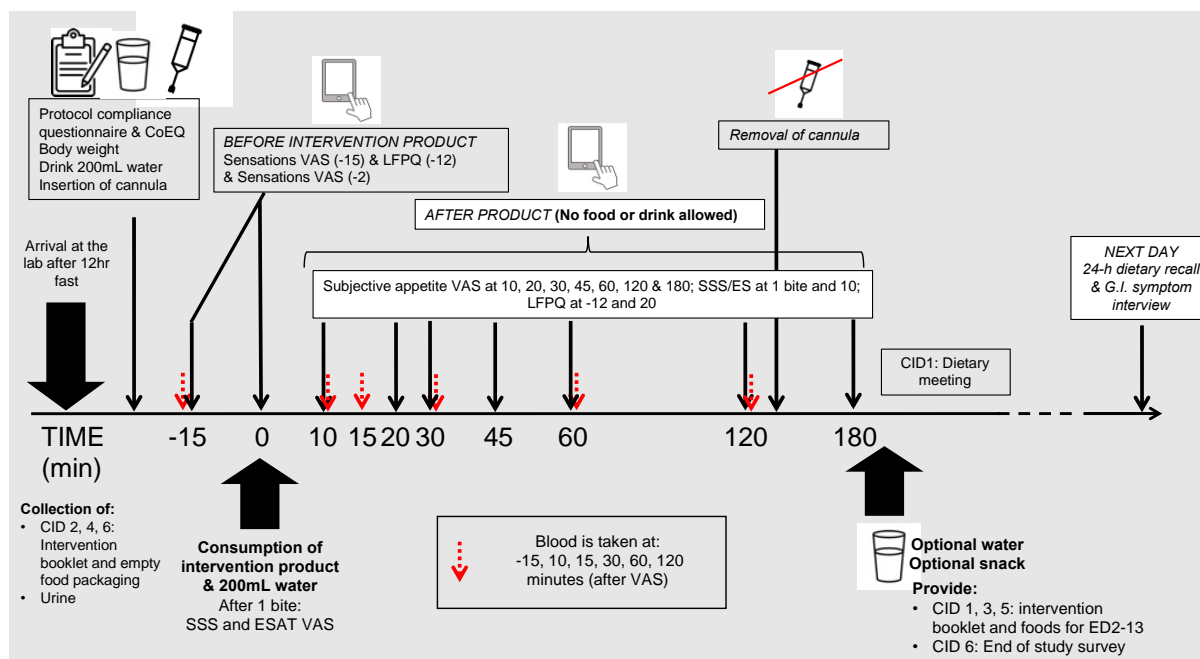


Figure 2: Example timeline of events during Clinical Investigation Day for biscuit matrix. CoEQ, Control of Eating Questionnaire; ED, Exposure Day; ESAT, Expected Satiety; G.I., gastrointestinal; LFPQ, Leeds Food Preference Questionnaire; SSS, Sensory-Specific Satiety; VAS, Visual Analogue Scale.

Supplemental Material 1: Exclusion Criteria

General Criteria

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

Medical conditions as known by the person

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

Medication

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

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

- 10 • Cholesterol lowering medication, if the dose has changed during the last 3 months (i.e.,
11 the medication is allowed if the participant has been on a stable dose for at least 3 months).
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Supplemental Material 2: Product Nutritional Information

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

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

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2					
3	Sugars (g)	12.03	22.26	5.02	9.28
4	Polyols (g)	0	0	4.86	9.00
5	Fibre (g)	0.86	1.60	6.64	12.28
6	Proteins (g)	4.38	8.11	4.21	7.79
7	Salt (mg)	0.35	0.64	0.33	0.60
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Supplementary Material 3 – Energy Intake Calculation

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

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

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

1. Zandstra, E. H., Mathey, M. F., Graaf, C., & van Staveren, W. A. (2000). Short-term regulation of food intake in children, young adults and the elderly. *European Journal of Clinical Nutrition*, 54(3), 239-246. <http://www.ncbi.nlm.nih.gov/pubmed/10713747>
2. Almiron-Roig, E., Palla, L., Guest, K., Ricchiutu, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews*, 71(7), 458-473. <https://doi.org/10.1111/nure.12048>

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3 **Supplemental Material 4: Questions used to assess product taste text, subjective**
4 **appetite sensations, sensory specific satiety and expected satiety**
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8 **Food Taste Test (FTT) (Conducted at screening)**
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11 **Screen 1**
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13 You will now be presented with a food that we will ask you to evaluate. Please follow the
14 instructions as they appear on the screen.
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22 **Screen 2**
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- 24
25 1. Take a mouthful of the food provided.
26 2. Chew while counting to 5.
27 3. Swallow.
28 4. Answer the question by moving the arrow to the left or to the right.
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31 **How pleasant was this food?**
32 Not at all pleasant Extremely
33 pleasant
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40 -----
41 **Screen 3**
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43 Thank you. This is the end of the taste test.
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45 Please call the investigator after submitting your answer.
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Subjective Appetite Questions (used during all CIDs)

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

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

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

After 1 bite:

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

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

At 10 minutes:

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

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

After 1 bite:

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

At 10 minutes after full consumption and after SSS rating:

How much will this food stop you from feeling hungry between meals?

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

Calculation of Fatty Liver Index:

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

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

Calculation of Triglyceride Glucose Index:

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

$$\text{TyG index} = \text{Ln} [(\text{fasting triglycerides}) (\text{mg/dL}) \times \text{fasting glucose} (\text{mg/dL})] / 2$$

1. Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006). The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterology*, 6(1), 33. <https://doi.org/10.1186/1471-230X-6-33>
2. Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, 6(4), 299–304. <https://doi.org/10.1089/met.2008.0034>

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

Supplemental Material 6: Trial Registration Data Set

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

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3	treatment of hypothyroidism is allowed if the person has been on a stable dose for at least 3 months. Cholesterol lowering
4	medication, if the dose has changed during the last 3 months (i.e., the medication is allowed if the participant has been on a stable
5	dose for at least 3 months)
6	Interventional
7	Allocation: Randomised. Double-blind, within-subjects, cross-over trial
8	
9	Study type
10	Primary purpose: Evaluation
11	Phase: N/A
12	Date of first enrolment
13	April 2021
14	Target sample size
15	213
16	Recruitment status
17	Recruiting
18	Primary outcome(s)
19	Incremental area under the curve (iAUC) for composite appetite sensations in response to each product.
20	Leeds Food Preference Questionnaire (LFPQ) Explicit Liking, Implicit Wanting, Relative preference, Explicit wanting; Control of
21	Eating Questionnaire (CoEQ): Craving Control, Craving for Sweet, Craving for Savoury, Positive Mood; Blood Glucose
22	Incremental Area Under the Curve; Blood Insulin Incremental Area Under the Curve; Cephalic and intestinal satiety biomarkers:
23	Glucagon-like peptide-1 (GLP-1) Incremental area under the curve for blood GLP-1 concentrations in response to each product
24	(120 min post intake); Ghrelin Incremental area under the curve for blood Ghrelin concentrations in response to each product
25	(120 min post intake).
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Appendices: Informed Consent Materials and GDPR



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Informed Consent Form

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

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

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

Appendices: Informed Consent Materials and GDPR

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

Participant name: _____

Date: _____ Signature: _____

Appendices: Informed Consent Materials and GDPR

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

Consent from the study staff that provided the oral information:

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

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

Study staff name: _____

Date: _____ Signature: _____

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



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General Data Protection Regulation (GDPR) information for study participants

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

Appendices: Informed Consent Materials and GDPR

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

1. Which data will be collected and how?

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

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

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

2. How will my data be stored?

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

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

Appendices: Informed Consent Materials and GDPR

3. How long will my data be stored for?

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

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

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

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

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

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

Appendices: Informed Consent Materials and GDPR

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

5. How will my data be used?

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

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

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

6. Who will have access to my data?

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

Appendices: Informed Consent Materials and GDPR

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

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

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

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

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

8. How will my data be destroyed?

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

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

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

Appendices: Informed Consent Materials and GDPR

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

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

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

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

Participant's signature:

Date
Signature

Name

Researcher's signature:

Date
Signature

Name

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

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

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

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

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

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

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

1	Roles and	#5b	Name and contact information for the trial	15
2	responsibilities:		sponsor	
3	sponsor contact			
4	information			
5				
6				
7	Roles and	#5c	Role of study sponsor and funders, if any, in	15
8	responsibilities:		study design; collection, management, analysis,	
9	sponsor and funder		and interpretation of data; writing of the report;	
10			and the decision to submit the report for	
11			publication, including whether they will have	
12			ultimate authority over any of these activities	
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17	Roles and	#5d	Composition, roles, and responsibilities of the	7 and 15
18	responsibilities:		coordinating centre, steering committee,	
19	committees		endpoint adjudication committee, data	
20			management team, and other individuals or	
21			groups overseeing the trial, if applicable (see	
22			Item 21a for data monitoring committee)	
23				
24				
25				
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27	Introduction			
28				
29	Background and	#6a	Description of research question and justification	4
30	rationale		for undertaking the trial, including summary of	
31			relevant studies (published and unpublished)	
32			examining benefits and harms for each	
33			intervention	
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38	Background and	#6b	Explanation for choice of comparators	4
39	rationale: choice of			
40	comparators			
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43	Objectives	#7	Specific objectives or hypotheses	5
44				
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46	Trial design	#8	Description of trial design including type of trial	6
47			(eg, parallel group, crossover, factorial, single	
48			group), allocation ratio, and framework (eg,	
49			superiority, equivalence, non-inferiority,	
50			exploratory)	
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**Methods:
Participants,
interventions, and
outcomes**

1	Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
2				
3				
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8	Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7 & Supplemental Material 1
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14	Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7-8
15				
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20	Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	N/A not required
21				
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27	Interventions: adherence	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	N/A Not required
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34	Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A participants are not patients
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37	Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	12
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50	Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	7
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1	Sample size	#14	Estimated number of participants needed to	6
2			achieve study objectives and how it was	
3			determined, including clinical and statistical	
4			assumptions supporting any sample size	
5			calculations	
6				
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9	Recruitment	#15	Strategies for achieving adequate participant	8
10			enrolment to reach target sample size	
11				
12				
13	Methods:			
14	Assignment of			
15	interventions (for			
16	controlled trials)			
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20	Allocation: sequence	#16a	Method of generating the allocation sequence	8-9
21	generation		(eg, computer-generated random numbers), and	
22			list of any factors for stratification. To reduce	
23			predictability of a random sequence, details of	
24			any planned restriction (eg, blocking) should be	
25			provided in a separate document that is	
26			unavailable to those who enrol participants or	
27			assign interventions	
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33	Allocation	#16b	Mechanism of implementing the allocation	9
34	concealment		sequence (eg, central telephone; sequentially	
35	mechanism		numbered, opaque, sealed envelopes),	
36			describing any steps to conceal the sequence	
37			until interventions are assigned	
38				
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41	Allocation:	#16c	Who will generate the allocation sequence, who	8
42	implementation		will enrol participants, and who will assign	
43			participants to interventions	
44				
45				
46	Blinding (masking)	#17a	Who will be blinded after assignment to	9
47			interventions (eg, trial participants, care	
48			providers, outcome assessors, data analysts),	
49			and how	
50				
51				
52				
53	Blinding (masking):	#17b	If blinded, circumstances under which unblinding	N/A: No safety/critical
54	emergency		is permissible, and procedure for revealing a	efficacy issues present
55	unblinding		participant's allocated intervention during the trial	
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1 **Methods: Data**
 2 **collection,**
 3 **management, and**
 4 **analysis**
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8	Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol
9			15; Supplemental
10			Material 4
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22	Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols
23			N/A Data from
24			participants who
25			discontinue/ deviate from
26			protocol not collected
27			
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30	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
31			15; appendices
32			
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40	Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol
41			14
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47	Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)
48			N/A, no analysis
49			included
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51	Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)
52			N/A, no analysis
53			included
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Methods:**Monitoring**

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5	Data monitoring:	#21a	Composition of data monitoring committee
6	formal committee		(DMC); summary of its role and reporting
7			structure; statement of whether it is independent
8			from the sponsor and competing interests; and
9			reference to where further details about its
10			charter can be found, if not in the protocol.
11			Alternatively, an explanation of why a DMC is
12			not needed
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18	Data monitoring:	#21b	Description of any interim analyses and stopping
19	interim analysis		guidelines, including who will have access to
20			these interim results and make the final decision
21			to terminate the trial
22			
23			
24	Harms	#22	Plans for collecting, assessing, reporting, and
25			managing solicited and spontaneously reported
26			adverse events and other unintended effects of
27			trial interventions or trial conduct
28			
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31	Auditing	#23	Frequency and procedures for auditing trial
32			conduct, if any, and whether the process will be
33			independent from investigators and the sponsor
34			
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36			
37	Ethics and		
38	dissemination		
39			
40	Research ethics	#24	Plans for seeking research ethics committee /
41	approval		institutional review board (REC / IRB) approval
42			
43			
44	Protocol	#25	Plans for communicating important protocol
45	amendments		modifications (eg, changes to eligibility criteria,
46			outcomes, analyses) to relevant parties (eg,
47			investigators, REC / IRBs, trial participants, trial
48			registries, journals, regulators)
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52	Consent or assent	#26a	Who will obtain informed consent or assent from
53			potential trial participants or authorised
54			surrogates, and how (see Item 32)
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1	Consent or assent:	#26b	Additional consent provisions for collection and	N/A
2	ancillary studies		use of participant data and biological specimens	
3			in ancillary studies, if applicable	
4				
5				
6	Confidentiality	#27	How personal information about potential and	Appendices
7			enrolled participants will be collected, shared,	
8			and maintained in order to protect confidentiality	
9			before, during, and after the trial	
10				
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13	Declaration of	#28	Financial and other competing interests for	15
14	interests		principal investigators for the overall trial and	
15			each study site	
16				
17				
18	Data access	#29	Statement of who will have access to the final	15
19			trial dataset, and disclosure of contractual	
20			agreements that limit such access for	
21			investigators	
22				
23				
24				
25	Ancillary and post	#30	Provisions, if any, for ancillary and post-trial	N/A: No safety/critical
26	trial care		care, and for compensation to those who suffer	efficacy issues present
27			harm from trial participation	
28				
29				
30				
31	Dissemination	#31a	Plans for investigators and sponsor to	15
32	policy: trial results		communicate trial results to participants,	
33			healthcare professionals, the public, and other	
34			relevant groups (eg, via publication, reporting in	
35			results databases, or other data sharing	
36			arrangements), including any publication	
37			restrictions	
38				
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41				
42	Dissemination	#31b	Authorship eligibility guidelines and any intended	16
43	policy: authorship		use of professional writers	
44				
45				
46	Dissemination	#31c	Plans, if any, for granting public access to the full	15
47	policy: reproducible		protocol, participant-level dataset, and statistical	
48	research		code	
49				
50				
51	Appendices			
52				
53				
54	Informed consent	#32	Model consent form and other related	Appendices
55	materials		documentation given to participants and	
56			authorised surrogates	
57				
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1 Biological specimens [#33](#) Plans for collection, laboratory evaluation, and 12
2 storage of biological specimens for genetic or
3 molecular analysis in the current trial and for
4 future use in ancillary studies, if applicable
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10 <https://www.goodreports.org/>, a tool made by the [EQUATOR Network](#) in collaboration with
11 [Penelope.ai](#)
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