

1 **Impact of acute consumption of beverages containing plant-based or alternative sweetener**  
2 **blends on postprandial appetite, food intake, metabolism, and gastro-intestinal symptoms:**  
3 **results of the SWEET Beverages trial**

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32 **Abstract**

33 Project SWEET examined the barriers and facilitators to the use of non-nutritive sweeteners and  
34 sweetness enhancers (hereafter “S&SE”) alongside potential risks/benefits for health and  
35 sustainability. The Beverages trial was a double-blind multi-centre, randomised crossover trial  
36 within SWEET evaluating the acute impact of three S&SE blends (plant-based and alternatives)  
37 vs. a sucrose control on glycaemic response, food intake, appetite sensations and safety after a  
38 carbohydrate-rich breakfast meal. The blends were: mogroside V and stevia RebM; stevia RebA  
39 and thaumatin; and sucralose and acesulfame-potassium (ace-K). At each 4h visit, 60 healthy  
40 volunteers (53% male; all with overweight/obesity) consumed a 330 mL beverage with either an  
41 S&SE blend (0 kJ) or 8% sucrose (26 g, 442 kJ), shortly followed by a standardised breakfast (~2600  
42 or 1800 kJ with 77 or 51 g carbohydrates, depending on sex). All blends reduced the 2-h  
43 incremental area-under-the-curve (iAUC) for blood insulin ( $p<0.001$  in mixed-effects models),  
44 while the stevia RebA and sucralose blends reduced the glucose iAUC ( $p<0.05$ ) compared with  
45 sucrose. Post-prandial levels of triglycerides plus hepatic transaminases did not differ across  
46 conditions ( $p>0.05$  for all). Compared with sucrose, there was a 3% increase in LDL-cholesterol  
47 after stevia RebA-thaumatin ( $p<0.001$  in adjusted models); and a 2% decrease in HDL-cholesterol  
48 after sucralose-ace-K ( $p<0.01$ ). There was an impact of blend on fullness and desire to eat ratings  
49 (both  $p<0.05$ ) and sucralose-acesulfame K induced higher prospective intake vs sucrose ( $p<0.001$   
50 in adjusted models), but changes were of a small magnitude and did not translate into energy  
51 intake differences over the next 24h. Gastro-intestinal symptoms for all beverages were mostly  
52 mild. In general, responses to a carbohydrate-rich meal following consumption of S&SE blends  
53 with stevia or sucralose were similar to sucrose.

54 **Keywords:** insulin, sweetness enhancer, glycaemic response, satiety, lipids.

55

56 **1. INTRODUCTION**

57 Obesity is a major health problem adding to the global burden of disease. Sugar intake is one  
58 dietary component that has gained attention as a major contributor to the overall energy density  
59 of diets, with excess intake promoting weight gain (WHO, 2018). In 2015, the World Health  
60 Organization recommended that free sugar intake should constitute <10% of total daily energy  
61 intake (E%) and preferably <5 E% for optimised health (WHO, 2015). However, due to the  
62 palatability of sweet foods and their ubiquitous presence, a large part of the population does not  
63 comply with this recommendation. For example, in the UK, added sugars (excluding those found  
64 naturally in fruit, vegetables and milk) contribute about 10 E% (Public Health England, 2020),  
65 while in Denmark the average intake of free and/or added sugars is 10-16 E% (Nordic Council of  
66 Ministers., n.d.). In Spain, half of the total sugar consumption (average 17 E%) is estimated to be  
67 free sugars (which include sugars naturally present in foods) (Ruiz et al., 2017)(WHO, 2015).

68 Epidemiological data reveal that sugar-sweetened beverages (SSBs) are one major source of  
69 added sugar intake across all age groups (Malik & Hu, 2022; Singh et al., 2015). To reduce dietary  
70 sources of added sugars, one recommended approach is to consume water instead of SSBs  
71 (Ebbeling et al., 2012). Another strategy is to choose beverages containing low- or non-calorie  
72 sweeteners in place of sugar (i.e. sugar replacers or non-nutritive sweeteners and sweetness  
73 enhancers - S&SEs). S&SEs have been shown to provide desired sweetness with little to no  
74 calories and contribute to reduced energy intake plus potentially, to better weight management  
75 (Lee et al., 2021; Rios-Leyvraz & Montez, 2022). S&SEs have also shown beneficial effects on  
76 blood glucose control and are used in the management of diabetes (British Dietetic Association,  
77 2016; EFSA, 2011).

78 There is currently inconsistent evidence on the short-term effects of S&SE-containing products  
79 and limited data on the long-term effects, in particular on safety aspects and efficacy, with  
80 studies suggesting either benefits or adverse effects (Higgins & Mattes, 2019; Rios-Leyvraz &  
81 Montez, 2022; Suez et al., 2014; Sylvetsky & Rother, 2018). These controversies likely arise due  
82 to differences in study design and perhaps also because S&SE represent a variety of substances

83 that act in different ways and may not collectively share the same mechanisms of action. This is  
84 possibly linked to each sweetener's unique chemical structure (Buchanan et al., 2022; Dalenberg  
85 et al., 2020; Higgins & Mattes, 2019; Yunker et al., 2021). Recent work suggests altered neural  
86 food cue responsivity for some S&SEs (Yunker et al., 2021), highlighting that not all S&SEs behave  
87 equally.

88 While some sweeteners could potentially increase subjective appetite, short-term randomised  
89 controlled trials show a consistent reduction in energy intakes when S&SEs replace sugars,  
90 although the effects are typically associated to single S&SEs rather than blends (Lee et al., 2021;  
91 O'Connor et al., 2021; Rios-Leyvraz & Montez, 2022). Acute and long-term effects may also differ  
92 and the role of reverse causality in observational studies cannot be ruled out (Rios-Leyvraz &  
93 Montez, 2022; Rogers et al., 2016). Taken as a whole, there is currently insufficient evidence to  
94 determine the extent of any undesirable effects of particular S&SE and S&SE blends on appetite,  
95 glucose metabolism and safety parameters.

96 As part of SWEET (SWEET Project, 2019), this study employed a multi-centre trial involving an  
97 acute intervention to explore initial acceptance, safety and post-prandial effects of S&SE blends  
98 delivered in beverage form prior to a meal. An *a priori* approach with comprehensive selection  
99 criteria was used to determine which blends to include in the trial considering regulatory status,  
100 sensory attributes, food and beverage functionality, industry use, and market/consumer trends.  
101 The three selected blends were: stevia rebaudioside M 80% purity (RebM) and mogroside V 50%  
102 purity (luo han Guo, monk fruit extract); stevia rebaudioside A 95% purity (RebA) and thaumatin;  
103 and sucralose and acesulfame-potassium (ace-K). Stevia RebA and RebM are both steviol  
104 rebaudiosides from the *Stevia rebaudiana* plant, which exist at different concentrations. Stevia  
105 RebM is noted to have more sweetness and less bitterness than can be found in RebA which is  
106 the most widely used stevia. Mogroside V is also a glycoside extracted from the monk fruit plant  
107 (*Siraitia Grosvenorii*), while thaumatin is a sweet tasting protein derived from the African  
108 *Thaumatococcus daniellii* plant (Mora & Dando, 2021). To our knowledge, the stevia RebM and  
109 mogroside V blend is used commercially with limited global prevalence (but not necessarily in

110 the ratio used in SWEET); however, the stevia RebA and thaumatin blend is not and is therefore  
111 relatively novel.

112 The null hypothesis tested in the present study was that the consumption of beverages  
113 sweetened with S&SE blends prior to a carbohydrate-rich meal would not significantly affect  
114 responses (including glycaemic response markers) relative to sucrose. Acute effects of different  
115 S&SE blends on appetite sensations, food intake (including energy intake, energy compensation  
116 and prospective food intake), safety (including gastro-intestinal (GI) symptoms, lipid and hepatic  
117 markers), and initial acceptance, were also investigated.

118

## 119 **2. METHODS**

### 120 *2.1. STUDY DESIGN*

121 The study was designed as a double-blind, multicentre randomised cross-over acute intervention  
122 study across three European centres (Spain, Denmark and UK). Participants were recruited and  
123 involved in the study between August 2020 and June 2021 and the study was performed in line  
124 with the principles of the Declaration of Helsinki. Approval was granted by the corresponding  
125 Research Ethics Committees for Denmark, the University of Copenhagen (ref. H-19085058);  
126 Spain, the University of Navarra (ref. 2019.213 mod1); and UK, the University of Liverpool (ref.  
127 6273). All participants provided signed informed consent and were compensated for their time  
128 with the equivalent of between €100 and €200.

129 The trial was registered in ClinicalTrials.gov under registration number NCT04483180.

130 Each participant attended four laboratory sessions (Clinical Investigation Days; CIDs), where one  
131 of four beverages (three with S&SE blends and a sucrose control) was tested. Wash-out periods  
132 between sessions were 6-10 days, but longer periods (12-21 days) were allowed under special  
133 circumstances (e.g. COVID-19 diagnosis).

134 Participants were randomised to one of four sequences created by the University of Leeds, based  
135 on a balanced block design to ensure equal number of comparable subjects under each

136 treatment order at each centre. Each sequence of exposure was stratified by sex (female/male),  
137 and age group (18-45 years/46-60 years) and intervention site (UNAV, UCPH, ULIV). In addition,  
138 a female/male ratio of minimum 60/40 was considered to reflect the target population  
139 characteristics. The person responsible for generating the sequence did not have any study  
140 related tasks (e.g. inclusion or examination participants). Blinding of the beverages was applied  
141 by the manufacturers and both participants and researchers including the data analyst were  
142 blinded.

## 143 2.2. PARTICIPANTS

144 Participants were healthy men and women, aged 18-60 y, with overweight or obesity (BMI 25 to  
145 35 kg/m<sup>2</sup>), regular consumers of sugar-containing foods and drinks and willing to consume plant-  
146 based or alternative non-caloric sweeteners (i.e. from chemical synthesis). Furthermore,  
147 participants also had to consume breakfast  $\geq 5$  days/week and like the control beverage (sucrose).

148 Exclusion criteria included lifestyle habits (i.e. physical activity, eating out patterns), medical  
149 conditions and medication affecting appetite and body weight, GI health, sweetener intake and  
150 conduct of the study (further details in **Supplementary Material**).

151

## 152 2.3. PROCEDURES

### 153 2.3.1. Screening session

154 Written informed consent was obtained prior to the screening session in the laboratory. During  
155 screening, medical history and concomitant medication were registered, and body weight and  
156 height measured to verify BMI criteria. Lack of eating disorders was confirmed with the Eating  
157 Attitudes Test-26 (EAT-26) (Garner & Garfinkel, 1979) for which a score  $< 20$  was required. Hip  
158 and waist circumference and waist-to-hip ratio (WHR) were also measured. A short questionnaire  
159 was used to confirm that participants were habitual consumers of sweetened products and liked  
160 sweet beverages. Candidates also rated their liking for 50 mL of the control beverage on an  
161 electronic anchored line scale or VAS (visual analogue scale) (a score of  $\geq 40/100$  mm was  
162 required). All eligible candidates completed the International Physical Activity Questionnaire

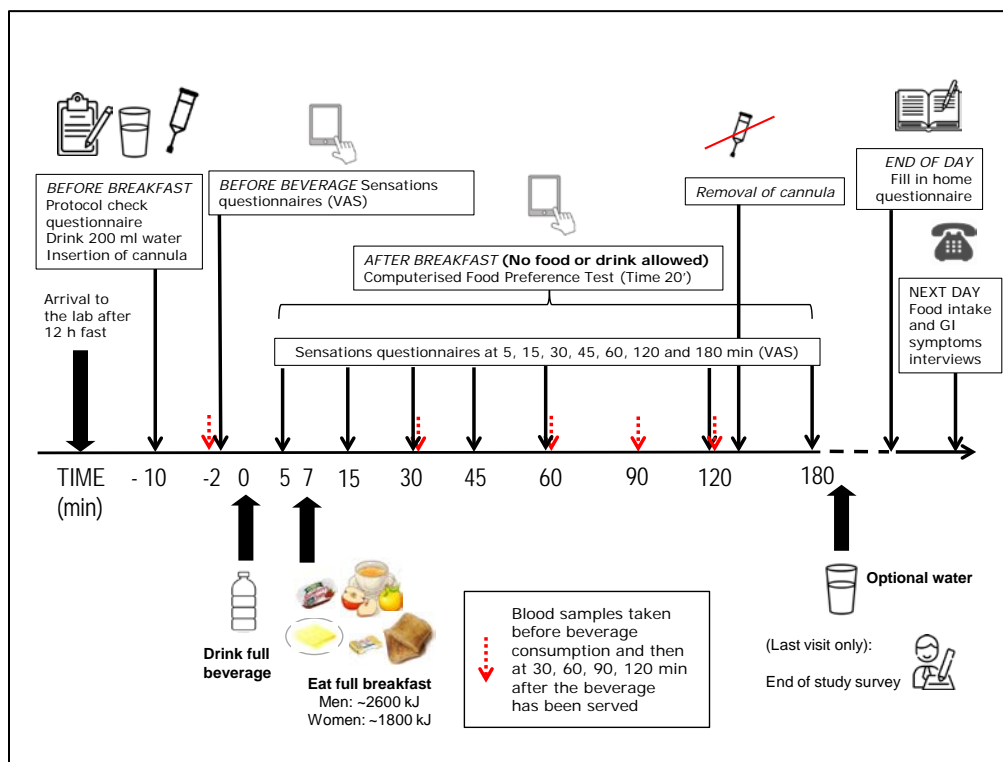
163 (IPAQ) (Booth, 2000) and a socio-demographic questionnaire (all questionnaires described  
164 below).

165

### 166 2.3.2. Clinical Investigation Days

167 **Figure 1** shows the procedures for the CIDs.

168 Prior to each CID, participants fasted for a minimum of 12 h (excluding up to 500 mL still water)  
169 and high-intensity physical activity, alcoholic beverages and coffee were not allowed for 12 h  
170 before arriving to the laboratory. These requirements were monitored at arrival and participants  
171 not complying with the protocol were scheduled for a later date (within a maximum of four days).



172

173 **Figure 1.** Clinical Investigation Day procedures. *Abbreviations:* GI, gastro-intestinal; VAS,  
174 visual analogue scale.

175

176 CID starting times were scheduled between 08:00 and 10:30 am, however participants had to  
177 attend at the same time on each CID. To standardise thirst levels, participants drank 200 mL water  
178 at arrival. On the last CID before drinking the water, participants were weighed in light clothing.  
179 Before participants saw the intervention foods, a cannula was inserted and after 10 min of resting  
180 a fasting blood sample was drawn. Following this, subjective appetite sensations, nausea and  
181 bloating (“sensations questionnaire” on Figure 1) were registered using electronic VAS.

182 One of the four beverages was then served and the participant was instructed to consume it all  
183 within 5 min (Time point 0 min). The participant then recorded appetite sensations, liking and  
184 desire for more beverage (Time point 5 min). Following this, participants consumed the complete  
185 breakfast within a maximum of 10 min. The breakfast consisted of customary items and was  
186 standardized across countries (see details below). For participants who refused to consume all  
187 the food, the reason and weight of any left-overs (measured covertly) were registered.

188 Participants remained seated in the intervention area completing questionnaires for a period of  
189 180 min, during which no food or beverage were allowed. The same sensations questionnaire  
190 (VAS) was completed at times ~15, 30, 45, 60, 120 and 180 min. In addition, at time 20 min  
191 participants completed the Leeds Food Preference Questionnaire (LFPQ) (reported separately).  
192 Postprandial blood samples were drawn at 30, 60, 90 and 120 min. Before leaving the laboratory,  
193 the participant received an End of Day questionnaire to register food cravings at home. On the  
194 next day participants undertook a telephone interview where GI symptoms plus all consumed  
195 foods and beverages between leaving the laboratory and until 24 h after consuming the test  
196 beverage on the CID were registered. On CID4, participants were offered to complete an End of  
197 study survey asking about the study design, treatment by staff, materials and compensation.

198

#### 199 2.4. BREAKFAST AND TEST BEVERAGES

200

201 **Table 1** lists the composition of each of the beverages used in the trials. Blends are hereafter  
202 referred to as: StM\_Mog (stevia RebM 80% purity and Mogroside V 50% purity); StA\_Tha (stevia



203 RebA 95% purity and thaumatin); Suc\_Ace (sucralose and ace-K). For plant extracts (stevia and  
204 mogroside) the purity ranges from 50% to 95% based on what is commercially available. The  
205 other sweeteners are synthesized (except for thaumatin which is a protein) and all are >95% pure.

206 The S&SE used have previously been approved for human consumption and have been granted  
207 EU or USA regulatory food status. The selected S&SE represented a diverse array including  
208 common commercial and consumer known sweetener blends, plus novel sweetener blends that  
209 have not been well studied yet, and were chosen based on their properties and/or existing data.

210 S&SE amounts were determined using the Beidler equation (prediction of sweetness intensities)  
211 (Graaf & Frijters, 1986; Schiffman et al., 2003), to match a sucrose equivalent (SEV) of 8%, an  
212 acceptable level chosen to represent the ranges of 5-12%, typically found in sugar sweetened  
213 beverages. An 8% SEV level can be matched with the use of S&SE and avoids inclusion of amounts  
214 of S&SE that can introduce bitter, metallic or off tastes.

215 Test beverages were all water-based, non-carbonated and lemon flavoured, supplied in identical  
216 330 mL clear, lidded bottles, labelled with a numerical code. Beverages were served in their  
217 original container alongside an empty 250 mL glass for optional use. The control, sucrose  
218 beverage (8% sucrose), provided 442 kJ (105.6 kcal) in total and contained 26.4 g sucrose (amount  
219 needed to produce a SEV of 8% in a volume of 330 ml). The three S&SE beverages provided 0 kJ.  
220 All four beverages were designed to be matched for sweetness intensity, flavour and physical  
221 appearance. Pre-study sensory analysis confirmed reasonable acceptance for all four  
222 intervention beverages (see Supplementary Material).

223 Crystalline sucrose and food grade stevia RebA and stevia RebM were obtained from Cargill B.V.,  
224 (Vilvoorde, Belgium). Mogroside V was purchased from Anderson Advanced Ingredients (Irvine,  
225 CA, USA). Thaumatin was kindly provided as a gift from Natex (Letchworth Garden City, UK). Food  
226 grade Ace-K was purchased from Sigma-Aldrich Inc. (St Louis, MO, USA) and sucralose was  
227 purchased from Prinova-Spectrum (London, UK). Shortly after consuming the S&SE beverages,  
228 male or female subjects consumed a standardized breakfast containing ~2600 or 1800 kJ and 77  
229 or 51 g glycemic carbohydrates, respectively. Nutrient and energy information for the breakfasts

230 is provided in **Table S1** in the Supplementary Material. All breakfast products were free from non-  
231 caloric and low-calorie sweeteners and were commercially available.

232

233 Table 1. Composition of the 330 mL test beverages (per 100 mL) by sweetener type.

Ingredients (in 100 mL)	StM_Mog	StA_Tha	Suc_Ace	Sucrose
Water (g)	94.77	94.81	94.82	86.83
Sucrose (g)	0	0	0	8.00
Mogroside V (g)	0.04	0	0	0
Stevia RebM (g)	0.02	0	0	0
Stevia RebA (g)	0	0.024	0	0
Thaumatococin (g)	0	0.00012	0	0
Sucralose (g)	0	0	0.01	0
Ace-K (g)	0	0	0.01	0
Potassium Citrate (g)	1.04	1.04	1.04	1.04
Citric Acid (g)	3.93	3.93	3.93	3.93
Sodium Benzoate (g)	0.02	0.02	0.02	0.02
Natural Lemon flavour (g)	0.18	0.18	0.18	0.18

234

235

## 236 2.5. DATA COLLECTION

### 237 2.5.1. Questionnaires

238 All common questionnaires were developed in English and translated to local languages. Where  
239 available, previously validated, translated versions for the corresponding study populations were  
240 preferentially used (i.e. Danish, Spanish). Questionnaires were delivered by the Questionnaire  
241 Delivery Platform (QDP), implemented by NetUnion (Lausanne, Switzerland), except for the  
242 LFPQ, implemented in E-Prime (Psychology Software Tools, Sharpsburg, PA, USA).

243 The sensations questionnaire consisted of a total of 11 electronic VAS related to pleasantness,  
244 desire for, appetite, satiety and G.I. symptoms and was administered using a tablet/PC with a link  
245 accessing the QDP. Validated questions for liking of the taste and desire for drinking more  
246 beverage, hunger, fullness, thirst, desire to eat, prospective intake, nausea, bloating, appetite for

247 something savoury and appetite for something sweet (Finlayson et al., 2008; Flint et al., 2000;  
248 Hill & Blundell, 1982) were shown on separate screens and the response was automatically  
249 registered standardised to 100 (based on a 100 mm VAS). Data for thirst, nausea, bloating,  
250 appetite for something savoury and for something sweet were all similar across conditions and  
251 are not reported further. The remaining set of appetite VAS (hunger, fullness, desire to eat and  
252 prospective food consumption) are referred to as “appetite sensations”. The full questionnaire  
253 can be accessed by contacting the authors.

254  
255 Additional questionnaires were used to measure habitual consumption of sweet foods, physical  
256 activity, socio-demographic characteristics, perceptions of the intervention (end of study survey  
257 in Fig. 1), food preference, food cravings and consumer S&SE perceptions (see Supplementary  
258 Material for details). The last 3 sets of data will be presented in a separate publication.

259  
260 2.5.2. Gastro-intestinal symptoms interview

261 The GI health assessment (presence of symptoms, duration and intensity) was carried out via a  
262 telephonic, standardised, 24-h interview using a tool based on the validated Gastro-Intestinal  
263 Symptom Rating Scale (Svedlund et al., 1988). Participants were asked about any experienced GI  
264 symptoms since they consumed the test beverage and up to 24 h later and to report whether  
265 they believed symptoms were associated with the test beverage. Any GI symptoms that had not  
266 been reported at screening were recorded as an adverse event.

267  
268 2.5.3. Dietary intake interview

269 Dietary assessment was carried out via a telephonic, standardised, 24-h recall (interview)  
270 following an adaptation of the validated 24-h recall method for NHANES (Centers for Disease  
271 Control and Prevention, n.d.). Participants were asked to verbally report everything they ate and  
272 drank (including recipe description and amounts) over the 24 h after drinking the test beverage

273 in the laboratory. To facilitate the interview, participants were allowed to take photographs  
274 and/or keep food packaging, and to use portion size measuring guides. The Australian Health  
275 Survey (AHS) food model booklet (Australian Bureau of Statistics, 2010), a piloted Danish food  
276 model booklet (Tjønneland et al., 2007) and the AHS plus the Young Persons Food atlases (Foster  
277 et al., 2017) were used in Spain, Denmark and the UK, respectively. The information from the 24-  
278 h food recall was converted to dietary intakes by using national nutrient composition data tables  
279 and software, specific to each country (Forestfield Software Ltd, 2021; Healthcare Software  
280 Solutions S.A., 2021; Kraftaerk Foodtech, n.d.).

281

## 282 *2.6. BLOOD SAMPLING AND PROCESSING*

283 Blood samples were only collected from Spanish (n=22) and Danish (n=20) participants due to  
284 unavailability of medical staff at the UK site caused by the COVID-19 pandemic.

285 Blood parameters analysed at each CID included glucose, insulin, lipid profile (triglycerides and  
286 total, HDL- plus LDL-cholesterol), and liver function markers (alanine aminotransferase (ALT),  
287 aspartate aminotransferase (AST), plus gamma-glutamyltransferase (GGT)). All processed  
288 samples were stored at -80°C until shipment and analysed at the Bioiatriki Central Laboratory in  
289 Athens, Greece. For details of sample collection procedures see Supplementary Materials.

290 All biochemistry analyses were performed using a HITACHI cobas 800c system/701 and the  
291 corresponding reagents (ROCHE). Insulin concentrations were determined by  
292 electrochemiluminescence immunoassay (ROCHE, Basel, Switzerland) using a HITACHI cobas  
293 e801 automated immunoassay system (ROCHE). Glucose concentrations were determined by the  
294 hexokinase test (enzymatic ultra-violet); triglycerides were determined by the enzymatic  
295 colorimetric method (end point); total cholesterol was determined by colorimetric, oxidase,  
296 esterase, and peroxidase analysis; HDL- and LDL-cholesterol were determined by homogeneous  
297 enzymatic colorimetric analyses (direct polyethylene glycol method for HDL-cholesterol); AST and  
298 ALT were determined by enzymatic colorimetric assays, and GGT by enzymatic colorimetric G

299 glutamyl-carboxy-nitroanilide according to the International Federation of Clinical Chemistry  
300 guidelines.

301

302

### 303 *2.7. DATA MANAGEMENT AND PROCESSING*

304 The majority of the data were collected electronically and uploaded onto a common datahub.  
305 Other data were collected using either an electronic case report form (e-CRF) (Xolomon Tree, SL,  
306 Madrid, Spain) or on paper CRFs and later entered into the e-CRF system.

307 The trapezoid method (Wolever et al., 1991) was used for calculation of the iAUC, excluding  
308 fasting values to remove bias or differences at baseline.

309 The triglyceride and glucose index (TyG), a marker of insulin resistance and metabolic syndrome;  
310 the homeostatic model assessment for insulin resistance (HOMA-IR) score; and the fatty liver  
311 index (FLI) were calculated as reported previously (Ascaso et al., 2001; Bedogni et al., 2006;  
312 Simental-Mendía et al., 2008).

313 Percent energy compensation (%EC) was derived from the dietary recall data and calculated as:

$$314 \quad \%EC = [ (EI_{\text{Low Calorie Preload}} - EI_{\text{Regular Preload}}) / |EP| ] * 100$$

315 Where EI= energy intake subsequent to eating the low calorie or the regular preload (in this case,  
316 beverage with sucrose). In this case, the energy consumed over the 24 h after preload  
317 administration (that is, excluding the breakfast and beverage); and |EP|= difference in the energy  
318 provided by each low-calorie preload vs the sucrose (control) condition, in absolute value  
319 (Almiron-Roig et al., 2013). See Supplementary Material for interpretation procedures applied.

320

321

### 322 *2.8. SAMPLE SIZE AND STATISTICAL ANALYSES*

323 Sample size was estimated based on previous literature on low-calorie sweeteners (Anton et al.,  
324 2010; Brandt et al., 2006; Green et al., 2001; Jiménez-Domínguez et al., 2015; Tey et al., 2017b)  
325 and on validation studies for subjective appetite scales (Almiron-Roig et al., 2009; Flint et al.,  
326 2000). These studies have used sample sizes of 12-48 participants. To detect a minimum  
327 difference of 8 mm in appetite ratings on a 100 mm VAS with 80% power, alpha 0.05, and a  
328 within-subject SD of 14.4 mm (Almiron-Roig et al., 2009), an overall sample of 54 participants  
329 was needed (Jones & Kenward, 2015). The 54 participants would also cover effect sizes for blood  
330 glucose and insulin (a minimum of 16 was needed) (Green et al., 2001), energy intake and  
331 compensation (Almiron-Roig & Drewnowski, 2003), liking and desire (Rogers & Hardman, 2015).

332 All study hypotheses as well as the analytic plan were specified prior to data collection, except  
333 when otherwise stated. This included sub-group analyses for men vs women, younger (18-45 y)  
334 vs. older (46-60 y) participants, and pre-obesity (BMI 25-29 kg/m<sup>2</sup>) vs obesity Class I participants  
335 (BMI 30-35 kg/m<sup>2</sup>), when applicable.

336 Data are presented as means  $\pm$  SD or SE as stated, for all continuous variables. Qualitative data  
337 are summarized with a narrative synthesis (e.g. observations related to adverse events).  
338 Incremental area under the curve (iAUC) for glucose, insulin and the TyG index was calculated  
339 using the trapezoid method (Wolever et al., 1991). For appetite ratings, the net incremental AUC  
340 (niAUC) was used to account for negative values (Brouns et al., 2005; Douglas & Leidy, 2019).

341 Extreme points were defined based on the literature (Kassambara, 2022) as values above {Q3 +  
342 3×IQR} or below {Q1 - 3×IQR} where Q1 and Q3 are the first and third quartile, respectively. IQR  
343 is the interquartile range (IQR = Q3 - Q1). Only extreme points (but not outliers) were excluded  
344 from analyses except for nausea ratings (no data were excluded as it contained a too large  
345 number of extreme points).

346 Change in body weight over the course of the intervention was analysed by paired-samples *t*-  
347 tests.

348 The impact of S&SE or sucrose condition (hereafter referred to as “blend”) on all outcome  
349 variables was analysed with linear mixed effects regression models including a random intercept  
350 to account for the repeated observations for each individual, and fitted using maximum  
351 likelihood estimation, likelihood ratio tests (REML). Fixed effects explored included blend and  
352 time when appropriate. All models were adjusted *a priori* for intervention site, sex, age group,  
353 and breakfast energy intake when applicable. Tukey’s post-hoc tests were applied to control the  
354 error rate for multiple pairwise comparisons between blends when an overall impact of blend  
355 was detected or suspected..

356 Effect sizes and 95% CIs were computed as Cohen’s *d* (Cohen, 1988) using a correction factor to  
357 account for the cross-over nature of the study (Lakens, 2013) and assuming a correlation of 0.8  
358 between visits (Robinson et al., 2014). Effect sizes were defined as trivial ( $d < 0.2$ ), small (0.2 to  
359 0.49), moderate (0.5 to 0.79) or strong ( $\geq 0.8$ ) (Cohen, 1988).

360 The potential presence of carry-over effects on appetite ratings was investigated by comparing  
361 mean 3-h niAUC ratings for hunger, fullness, desire to eat and prospective intake across the 4  
362 potential treatment orders with ANOVA. Sensitivity analyses were then performed on those  
363 variables where the mean ratings differed across treatment order.

364 Differences in beverage liking and desire were detected as part of the main results, therefore, a  
365 data-driven, post-hoc analysis was performed to rule out unplanned effects of desire/liking on  
366 main study variables (i.e. hunger, fullness, desire to eat, prospective intake, 24 h *ad libitum* and  
367 total energy intakes). All analyses were carried out using the R-language free software, RStudio  
368 2022.12.0+353 (R Project for Statistical Computing, [www.r-project.org](http://www.r-project.org)). Statistical significance  
369 was set at  $p < 0.05$  or  $p < 0.01$  for multiple comparisons.

370

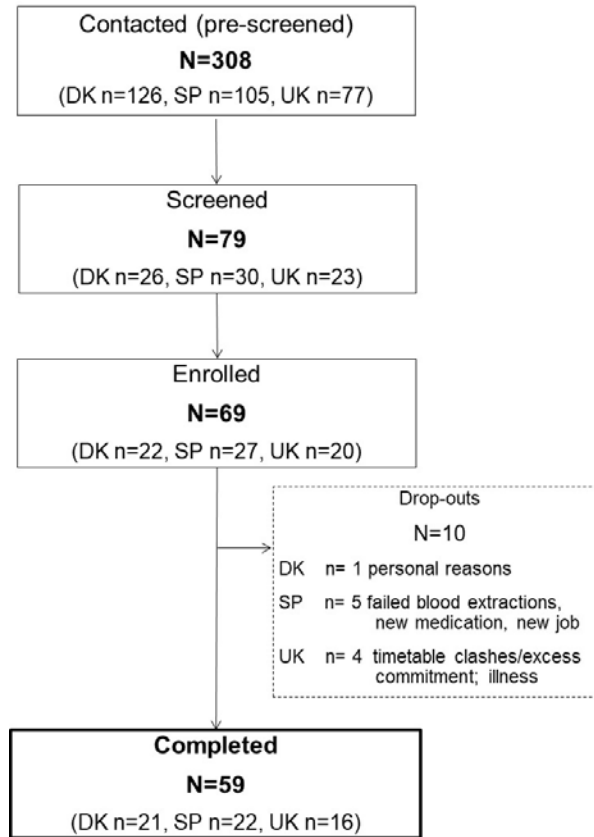
### 371 **3. RESULTS**

372 A total of 308 interested participants were contacted across the three sites of which 79 were  
373 screened and 69 were enrolled. Of those, 59 completed the four CIDs. There were 10 drop-outs  
374 in total, largely due to personal and medical reasons (**Figure 2**).

375



376



377

378 Figure 2. Recruitment flowchart for the Beverages multi-centre trial. Abbreviations: DK, Denmark  
379 (University of Copenhagen); SP, Spain (University of Navarra); UK, United Kingdom (University of  
380 Liverpool).

381

382 The analyses are based on participants completing the first visit i.e. CID1 (N=60). This sample is  
383 composed of 47% women and 53% men with a mean (SD) age and BMI of 32.1 (11.0) y and 28.9  
384 (2.8) kg/m<sup>2</sup> respectively. The distribution of anthropometric and other baseline data was similar  
385 across countries. Weight at the end of the study was not different from weight at baseline  
386 (p=0.405) (Table 2).

387 **Table 2.** Characteristics of participants completing CID1. Values are means (SD) unless otherwise  
 388 indicated. Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; EAT-26,  
 389 Eating attitudes test-26; IPAQ, International physical activity questionnaire; FL index, Fatty liver index;  
 390 GGT, Gamma-glutamyltransferase; TyG, Triglyceride and glucose index; VAS, Visual analogue scale;  
 391 WHR, waist-to-hip ratio; N/A: not applicable (no blood samples collected).

	All centres (N=60) <sup>a</sup>		Spain (n=22)		Denmark (n=21)		U.K. (n=17) <sup>a</sup>	
Sex								
Female (n)	28		11		8		9	
Male (n)	32		11		13		8	
Age (years)	32.1	(11.0)	33.5	(11.6)	33.1	(11.5)	28.9	(9.6)
Weight at baseline (kg)	85.9	(14.0)	80.3	(13.5)	93.1	(12.9)	84.3	(12.7)
Weight at study end (kg)	86.0	(14.0)	80.1	(13.6)	92.9	(13.0)	85.3	(12.6)
Height (cm)	171.5	(9.8)	168.1	(8.2)	177.0	(9.7)	169.6	(9.6)
BMI (kg/m <sup>2</sup> )	28.9	(2.8)	28.2	(2.7)	29.3	(2.6)	29.3	(3.3)
EAT-26 score (0-78)	5.4	(3.9)	6.5	(3.7)	4.5	(3.1)	5.1	(4.8)
Waist circumference (cm)	93.9	(12.1)	89.8	(13.4)	96.2	(10.9)	96.3	(11.0)
Hip circumference (cm)	108.7	(7.1)	107.0	(6.7)	108.5	(7.2)	111.1	(7.2)
WHR (cm)	0.86	(0.10)	0.84	(0.10)	0.89	(0.10)	0.87	(0.08)
Fasting glucose (mg/dL)	92.6	(6.5)	90.1	(6.1)	95.3	(5.9)	N/A	
Fasting insulin (μU/mL)	10.6	(5.5)	10.6	(5.9)	10.6	(5.1)	N/A	
Fasting triglycerides (mg/dL)	93.1	(45.6)	79.4	(32.0)	108.1	(53.9)	N/A	
Fasting total cholesterol (mg/dL)	165.9	(29.7)	172.1	(31.6)	159.1	(26.6)	N/A	
Fasting HDL-cholesterol (mg/dL)	52.9	(12.2)	56.6	(13.2)	48.9	(9.7)	N/A	
Fasting LDL-cholesterol (mg/dL)	102.5	(26.4)	107.7	(28.2)	96.8	(23.8)	N/A	
Fasting AST (IU/L)	23.4	(6.9)	24.1	(7.5)	22.6	(6.3)	N/A	
Fasting ALT (IU/L)	22.4	(13.9)	21.8	(11.9)	23.0	(16.1)	N/A	
Fasting GGT (IU/L)	25.8	(22.9)	30.1	(29.5)	21.1	(10.9)	N/A	
TyG index (cut off 4.65 points)	4.48	(0.2)	4.40	(0.2)	4.57	(0.2)	N/A	
FL index (cut off 60 points)	40	(27)	37	(29)	41	(26)	N/A	
HOMA-IR <sup>b</sup>	2.45	(1.3)	2.4	(1.4)	2.5	(1.3)	N/A	
Physical activity (IPAQ, Total MET-minutes/week) <sup>c</sup>	5636	(4531)	5068	(3595)	5809	(5116)	6110	(4932)
Habitual intake of sweet foods (short sugar FFQ score, 0-11)	8.3	(1.7)	7.2	(1.7)	9.1	(1.4)	8.8	(1.3)
Liking of control beverage (Taste test, 100 mm VAS)	80.5	(15.4)	82.6	(15.4)	77.2	(15.9)	81.8	(15.0)
Conduct of intervention (end of study survey score, 0-10)	9.30	(0.8)	9.46	(0.7)	8.81	(0.8)	9.71	(0.4)

392 <sup>a</sup> Includes one female who dropped out after CID3 due to illness (COVID-19 diagnosis).

393 <sup>b</sup> Cut-off value for HOMA-IR is 3.8 for healthy population and 2.1 for high risk population (Ascaso et al., 2001; Gayoso-Diz et al., 2013).

394 <sup>c</sup> Sample size for All centres N=45; Spain n=15; Denmark n=19; U.K. n=11.

396

397 The sample populations were  $\geq 75\%$  of white European descent, except in the UK where 35% were  
398 of East-Asian descent. Most participants in Spain and the UK reported holding or studying for a  
399 university-degree, while 48% of Danish participants reported secondary education as the highest  
400 level attained. One-third were employed full-time while 40% were on full-time education (**Table**  
401 **S2** in Supplementary information). Chronic-disease risk markers (waist circumference, WHR, TyG,  
402 FFI, and HOMA-IR) were overall within the healthy range or close (Bedogni et al., 2006; Gayoso-  
403 Diz et al., 2013; Simental-Mendía et al., 2008).

404

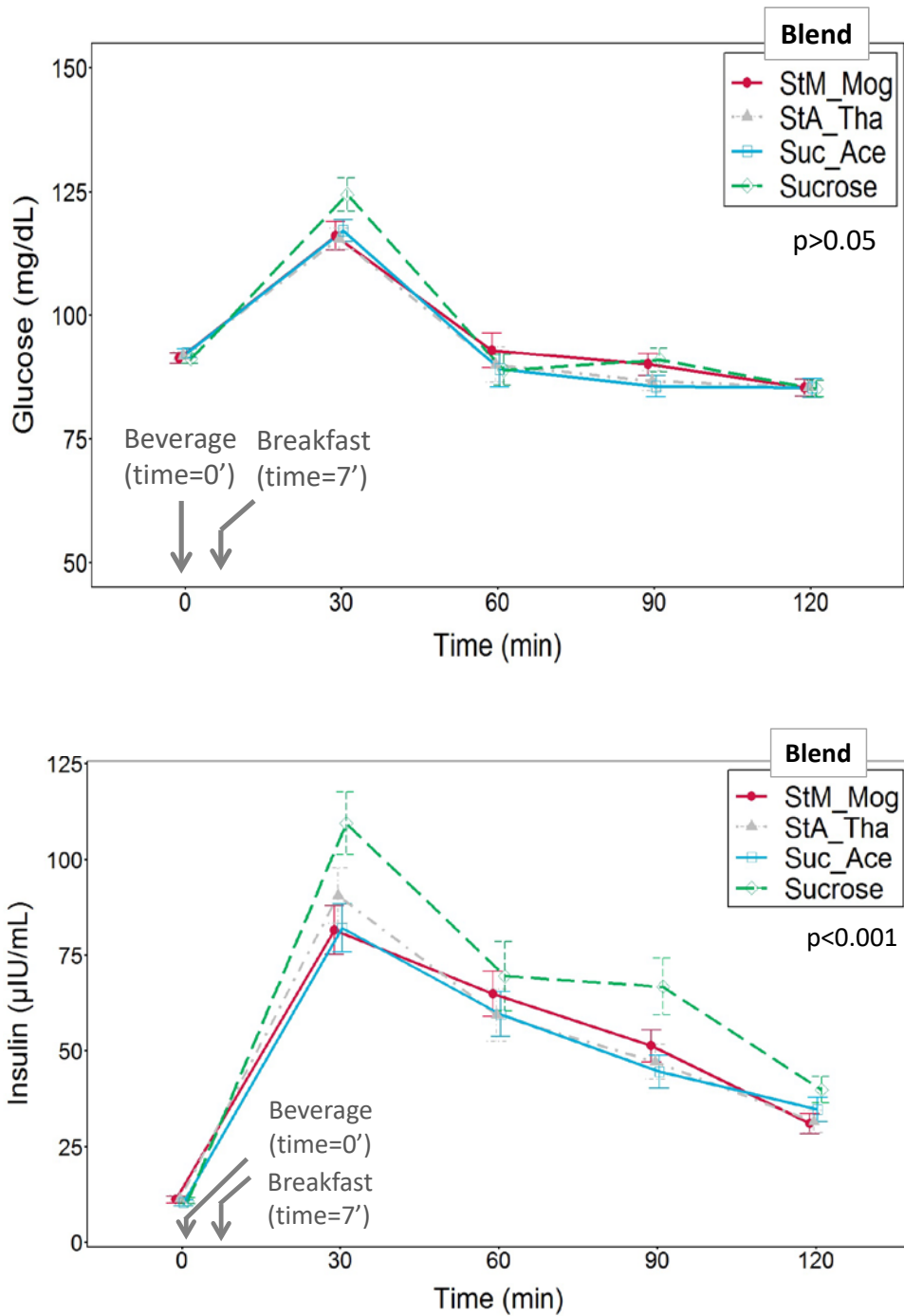
### 405 3.1. Glycaemic impact

406

407 There was an overall impact of blend on the 2-h iAUC for both glucose and insulin (**Table 3**).  
408 Calculated effect sizes (95%CI) for the glucose were small at best at -0.17 (-0.39, 0.04), -0.31 (-  
409 0.52, -0.09) and -0.32 (-0.53, -0.11) for the comparison of StM\_Mog, StA\_Tha and Suc\_Ace vs.  
410 sucrose, respectively. Insulin iAUC effect sizes were small at -0.39 (-0.60, -0.18), -0.40 (-0.62, -  
411 0.19) and -0.44 (-0.66, -0.22), respectively. Post-hoc Tukey's adjusted tests revealed significant  
412 differences in insulin iAUC for all three blends vs. sucrose ( $p < 0.001$  for all comparisons), but not  
413 for glucose iAUC ( $p > 0.01$ ). There were no differences between non-caloric blend pairs for either  
414 glucose nor insulin iAUCs ( $p > 0.05$  all comparisons). There was an impact of blend condition on  
415 the 2-h iAUC for the TyG index with StA\_Tha and Suc\_Ace reducing the TyG vs sucrose (overall  
416 effect of blend  $p < 0.05$ ), with trivial effect sizes (-0.17 to 0.01; 95%CI -0.38 to 0.23) (Table 3).

417 Post-prandial blood glucose and insulin levels are shown in **Figure 3**. In contrast with the AUC  
418 analysis, for glucose, the main effect of blend was non-significant ( $p = 0.286$ ). For insulin however,  
419 there was a significant impact of blend ( $p < 0.001$ ) and a Tukey's adjusted post-hoc analysis  
420 revealed lower concentrations after any of the S&SE blends vs. sucrose ( $p < 0.001$  for all  
421 comparisons), with no differences between non-caloric blend pairs.

422



424 Figure 3. Fasting and post-prandial blood glucose (top) and insulin levels (bottom) across blend condition (N=42).  
 425 Data points are means with SE. Overall impact of blend (linear mixed effects models results shown on the right  
 426 upper corner.

427

428 Table 3. Incremental area under the curve (iAUC) for glucose and insulin blood levels, and the triglyceride  
 429 and glucose index (TyG), after preload consumption (breakfast plus beverage). Values are mean (SD)  
 430 across centres.

		StM_Mog	StA_Tha	Suc_Ace	Sucrose	Overall impact of blend <sup>a</sup>
<b>Glucose iAUC</b> <b>(mg/dL x min)</b>	Mean	1132	985	967	1322	p=0.028
	(SD)	(1002)	(788)	(781)	(1144)	
N=42						
<b>Insulin iAUC</b> <b>(µU/mL x min)</b>	Mean	5120	5095	4965	6429	p=0.000
	(SD)	(2391)	(3015)	(2580)	(3480)	
N=42*						
<b>TyG Index iAUC</b> <b>(points x min)</b>	Mean	8.545	7.180	7.272	8.444	p=0.013
	(SD)	(8.670)	(6.548)	(5.872)	(7.742)	
N=42**						

431 <sup>a</sup> Linear mixed effects regression adjusted with intervention site, sex, age group and breakfast energy intake  
 432 (intervention site and sex remained significant in the final glucose and TyG models).

433 \*An extreme value was detected for StA\_Tha and for sucrose; plus, two for Suc\_Ace. These values were excluded.

434 \*\*An extreme value was detected for Suc\_Ace and this value was excluded.

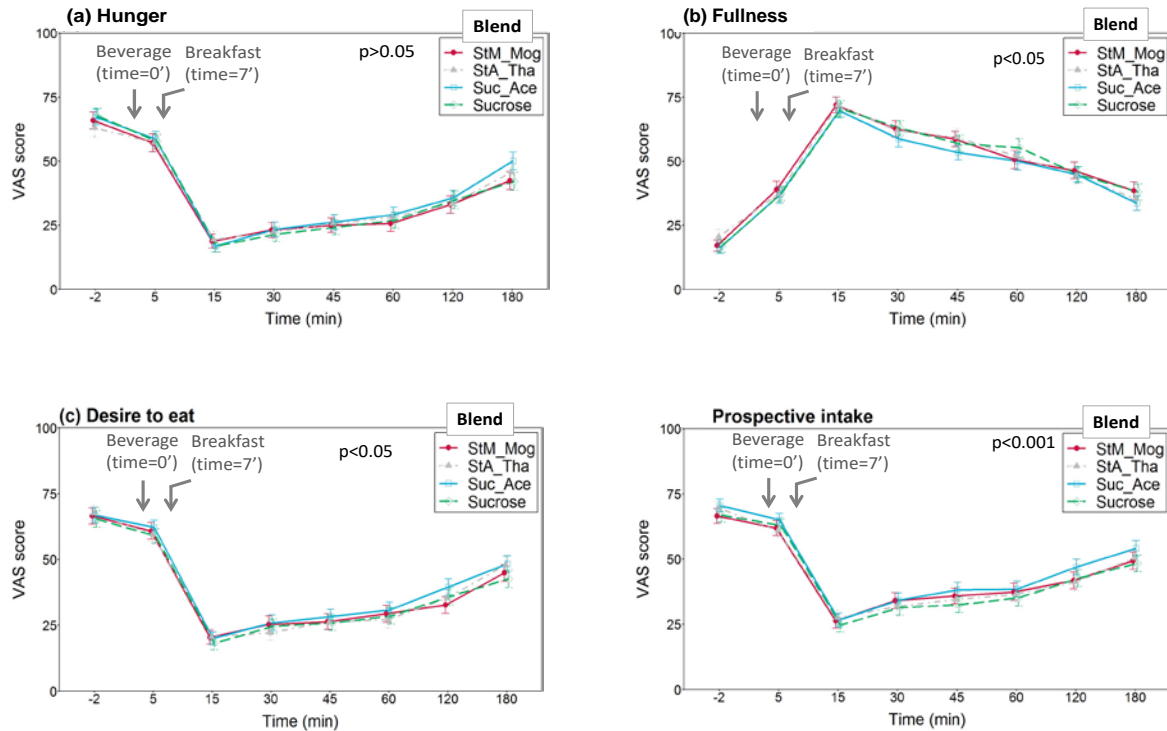
435 Mean values and details of B coefficients for the glucose and insulin 2-h iAUC models can be  
 436 found in supplementary **Tables S3 and S4**. Sex and intervention site remained significant  
 437 covariates in the glucose but not in the insulin models (see Supplementary Materials for details,  
 438 Additional Results).

439

### 440 3.2. Appetite response

441 **Figure 4.** shows the temporal profiles for subjective hunger, fullness, desire to eat and  
 442 prospective intake ratings across blend condition.

443



444 Figure 4. Temporal profiles for subjective hunger, fullness, desire to eat and prospective intake across  
 445 blend condition (N=58-60). Data points are means with SE. Overall impact of blend shown on the right  
 446 upper corner.

447

448 Different effects of the S&SE blends on the appetite response were detected. While there was  
 449 no major impact of any of the preloads containing S&SE over sucrose on appetite ratings, the  
 450 Suc\_Ace blend performed differently. In particular, the Suc\_Ace blend elicited higher prospective  
 451 intake sensations than the StA\_Tha blend and the sucrose (both  $p < 0.001$ ). An overall impact of  
 452 blend was also detected for desire to eat and fullness ratings (both  $p < 0.05$ ). These effects were  
 453 all of small magnitude. Indeed, hunger, fullness, desire to eat and prospective intake effect sizes  
 454 calculated using the 3-h niAUC were all trivial ( $d < 0.22$ ) despite differences seen in the 3-h curves

455 In terms of “rebound” hunger, there was no increase in the 2-h niAUC for hunger after any of the  
 456 S&SE conditions compared with sucrose ( $p = 0.442$ ).

457 Treatment order effects were detected only for fullness ( $p < 0.001$ ). Including treatment order as  
 458 covariate in the model for fullness ratings did not change the results.

459 3.3. Beverage liking and desire for more beverage scores

460

461 There were significant differences in both liking and desire ratings across blends ( $p < 0.001$  for  
 462 both models). Post-hoc analyses (Tukey’s- adjusted) confirmed that the sucrose and Suc\_Ace-  
 463 containing beverages were more liked and desired than both stevia-containing beverages (**Table**  
 464 **4**). Effect sizes (95%CI) for liking scores of each S&SE blend vs. sucrose ranged from trivial to  
 465 moderate: StM\_Mog -0.67 (-0.85, -0.49); StA\_Tha -0.60 (-0.79, -0.42) and Suc\_Ace -0.12 (-0.30,  
 466 0.06). For desire scores, effect sizes were similar: StM\_Mog -0.53 (-0.71, -0.36); StA\_Tha -0.63 (-  
 467 0.81, -0.45) and Suc\_Ace -0.15 (-0.32, 0.03).

468 Exploratory post-hoc tests for the influence of *liking* and *desire* revealed no significant effect of  
 469 either *liking* or *desire* on hunger and prospective intake ratings, nor on energy intake outcomes.  
 470 Desire for more beverage attenuated the impact of blend on fullness and desire to eat ratings,  
 471 while liking (pleasantness) attenuated the impact on fullness ratings (further details included in  
 472 Supplementary Material, Additional Results).

473

474 **Table 4.** Liking and desire scores for the intervention beverages collected at time 5 min (after drink  
 475 consumption). Values are mean (SD) across all centres. Means with different superscript letters differ at  
 476 the  $p < 0.001$  level (liking) or  $p < 0.05$  level (desire).

VAS rating (0-100 mm)	N*	StM_Mog	StA_Tha	Suc_Ace	Sucrose	Overall impact of blend
Liking <sup>#</sup>	59-60	59.53 (21.78) <sup>a</sup>	59.51 (23.95) <sup>a</sup>	71.32 (18.12) <sup>b</sup>	73.41 (16.90) <sup>b</sup>	$p < 0.001$
Desire <sup>§</sup>	58-60	34.75 (23.12) <sup>a</sup>	32.97 (21.53) <sup>a</sup>	43.5 (25.62) <sup>b</sup>	47.07 (23.07) <sup>c</sup>	$p < 0.001$

477 \* One outlier was identified and excluded for sucrose for “Liking”; two missing values for “Desire” and one missing  
 478 value for “Liking” (both for StA\_Tha) were identified and those subjects were excluded.

479 <sup>#</sup> Linear mixed effects regression adjusted for intervention site, age group, and sex. Intervention site and sex  
 480 retained a significant impact in the final model.

481 <sup>§</sup> Linear mixed effects regression adjusted for intervention site, age group and sex All covariates retained a  
 482 significant impact in the final model (see Supplementary Material, Additional Results, for details).

483

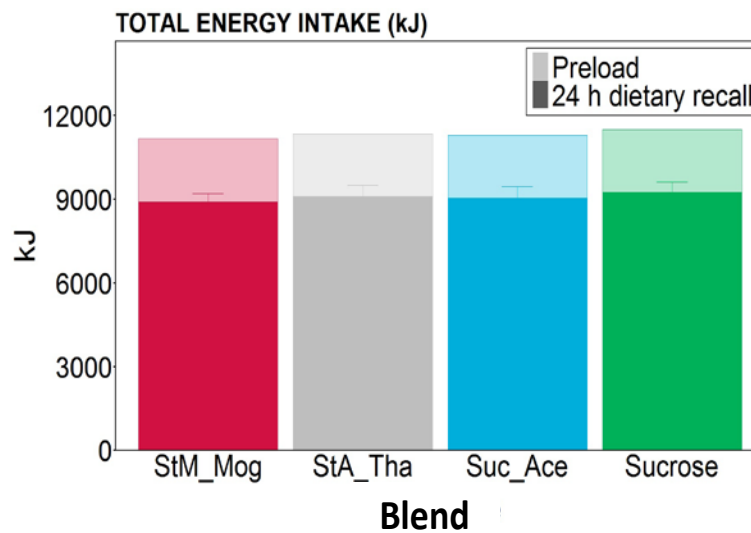
484

### 485 3.4. Energy and macronutrient intake

486 There were no blend-associated differences in *ad libitum* energy intake over the next 24 h or in  
487 total energy intake (including additionally the breakfast and beverage) ( $p>0.05$  both) (**Figure 5**).

488 Mean (SD) total energy intakes by blend were: StM\_Mog 11152 (86384) kJ; StA\_Tha 11321  
489 (86957) kJ; Suc\_Ace 11283 (87399) kJ; sucrose 11480 (88922) kJ. These values were not  
490 statistically different in adjusted models, with the maximum difference corresponding to around  
491 328 kJ (78 kcal) between the StM\_Mog and the sucrose conditions.

492



493

494 **Figure 5.** Total energy intake by blend condition. Data across all centres (N=59-60). Columns are total  
495 mean $\pm$ SE energy consumed including preload (beverage plus breakfast) and 24 h *ad libitum* intake. There  
496 were no significant differences across blend in 24h *ad libitum* energy intake ( $p=0.278$ ), or in total energy  
497 intakes ( $p=0.825$ ) in the adjusted models.

498



499 There was a significant impact of intervention site and sex (both  $p < 0.01$ ), on both 24h and total  
500 energy intakes. As expected, men consumed more total energy. Also, Spanish participants  
501 consumed less total energy than British and Danish ones.

502

503

#### 504 *3.4.1. Energy compensation*

505 Taking as reference the sucrose condition, no significant differences in percent energy  
506 compensation were detected across adjusted means (effect of blend  $p = 0.214$ ).

507

#### 508 *3.4.2. Twenty-four h ad libitum macronutrient intake*

509 Analysis of the 24 h dietary recall data revealed no significant impact of beverage on nutrient  
510 intakes over the 24 h period following preload consumption in adjusted models. However,  
511 intervention site remained a significant variable in all carbohydrate models (total carb, fibre and  
512 sugar), while sex remained a significant variable in the fat models (total fat, saturated and  
513 unsaturated fat intake).

514

### 515 3.5. Safety parameters

516

#### 517 *3.5.1. Blood lipids*

518 There was a small impact of some S&SE blends on some blood lipids, however, changes were of  
519 a very small magnitude (**Table S5**). Adjusted models showed an overall impact of blend on total  
520 and LDL-cholesterol (both  $p < 0.001$ ); and on HDL-cholesterol ( $p < 0.01$ ), but not on triglycerides  
521 ( $p = 0.371$ ). StA\_Tha increased LDL-cholesterol levels by 2.9% vs sucrose ( $p < 0.001$ ), and increased  
522 total cholesterol vs StM\_Mog ( $p < 0.001$ ) but not vs. sucrose ( $p = 0.076$ ). Also, compared with  
523 sucrose, all three S&SE blends reduced HDL-cholesterol by between 1.9 and 2.3% but the

524 reduction was only significant for Suc\_Ace (-2.3%,  $p < 0.01$ ). These small effects were not due to  
525 differences in fasting values ( $p > 0.05$  all comparisons).

526

### 527 *3.5.2. GI symptoms, other adverse events and medication*

528 There were no serious adverse events and most reported GI symptoms were mild although some  
529 were more frequent/intense such as belching, rumbling and altered frequency of opening  
530 bowels. Changes in concomitant medication during the study were accounted for in the analyses  
531 as was the presence of adverse events. There were no changes in medication that related to  
532 study procedures. Overall, no beverage was associated with important undesired metabolic or  
533 behavioural outcomes and there were no drop-outs related to adverse events.

534

## 535 **4. DISCUSSION**

536 The results of this study show that a range of plant-based and alternative sweeteners were  
537 comparable to sucrose in their metabolic effects after acute consumption in liquid form. Despite  
538 the co-ingestion of the beverages with a standardised breakfast, blood insulin rose higher after  
539 the sucrose vs all S&SE blends, suggesting an attenuation effect of the breakfast-induced insulin  
540 peak with all three S&SE blends. As expected, glucose and insulin iAUC values were higher after  
541 sucrose consumption, however differences in the 2-h glucose curve were not detectable,  
542 probably attenuated by the carbohydrate content of the breakfasts. On the other hand, in this  
543 study different S&SEs exerted different effects on subjective appetite sensations. Despite being  
544 similarly accepted as the energy-containing control, the Suc\_Ace blend was associated with a  
545 weaker satiety impact over 3 h vs sucrose. Specifically, the Suc\_Ace blend induced higher  
546 prospective intake vs the StA\_Tha blend and vs. sucrose, but changes were of a small magnitude  
547 and did not translate into energy intake differences over the next 24h.

548 Although there were effects of some of the blends on blood cholesterol levels, such effects were  
549 of very small magnitude (<3% vs the control condition in all cases). For reference, such changes

550 need to be of 10% or more to be considered clinically relevant in chronic interventions (American  
551 Diabetes Association, 2008; Bradley et al., 2009). We believe lipid changes in our study probably  
552 reflect spontaneous fluctuations not detectable at baseline. This is confirmed by a recent meta-  
553 analysis (Movahedian et al., 2021) and previous studies with S&SEs showing no effects on blood  
554 lipids in several diverse populations and when used in different doses over several months  
555 (Higgins & Mattes, 2019). LDL-cholesterol increases after consuming StA\_Tha in this study (about  
556 3 mg/dL vs. sucrose) were smaller compared with those reported in the literature (>4 mg/dL)  
557 (Movahedian et al., 2021). Despite this, the possibility that these effects may be cumulative or  
558 depend exclusively on the participant's BMI cannot be ruled out and so further investigation is  
559 needed.

560 In agreement with previous work related to the absence of adverse effects of S&SE on metabolic  
561 parameters (Gallagher et al., 2021; Movahedian et al., 2021; Nichol et al., 2018), none of the  
562 blends tested in the present study induced rebound hunger and all were safe in terms of hepatic  
563 impact and side effects. Our findings also confirm previous work related to the lack of adverse  
564 effects of S&SE on acute blood glucose control (Greylling et al., 2020; Tucker & Tan, 2017)

565 The present work revealed improved insulinemic responses for steviol glycosides and mogroside  
566 V vs sucrose. Several studies have examined the glycaemic impact of steviol glycosides, mostly  
567 stevia RebA (Anton et al., 2010; Stamataki, Crooks, et al., 2020; Stamataki, Scott, et al., 2020; Tey  
568 et al., 2017a, 2017b); and sucralose, with or without ace-K (Bryant et al., 2014; Pepino et al.,  
569 2013; Sylvetsky et al., 2016), with fewer studies evaluating mogroside V (Tey et al., 2017a,  
570 2017b). To the best of our knowledge, no peer-reviewed, comparable randomised clinical trial  
571 for thaumatin has been published.

572 The effects of stevia (as steviol glycosides, mostly RebA) are well documented and tend to agree  
573 with our results. Trials using stevia RebA in beverage form have shown improvements in the  
574 glycaemic response vs caloric (sucrose or glucose) preloads in acute settings (Stamataki, Scott, et  
575 al., 2020; Tey et al., 2017b), but no impact on the long-term, despite reductions in energy intakes  
576 (Stamataki, Crooks, et al., 2020; Tey et al., 2017a). In terms of the glycaemic response to a meal,

577 stevia RebA, but not other S&SEs, was found to attenuate the post-prandial blood glucose peak  
578 in previous studies (Anton et al., 2010; Stamataki, Scott, et al., 2020). We did not detect changes  
579 in the 2-h temporal profile of glucose after consumption of S&SEs or sucrose with a meal,  
580 however, all S&SE blends improved the 2- h insulin curve and both insulin and glucose iAUCs, vs.  
581 sucrose. The lack of impact of blends on the 2-h glucose curve is probably due to the relatively  
582 large carbohydrate load given very close to the meal with all beverages.

583

584 The main contrast between the present study and previous ones employing stevia is in the total  
585 energy intakes (including preload and *ad libitum* intake). While in a previous acute study  
586 (Stamataki, Scott, et al., 2020) participants ate overall less energy after a stevia RebA vs sucrose  
587 preload, in our study the reduction in total intakes for both blends including stevia (RebA and  
588 RebM) was more subtle and not significant. The results from Stamataki et al. 2020 also contrast  
589 with those from Anton et al., who used solid preloads sweetened with either stevia, aspartame,  
590 or sucrose, and found no added energy intake after S&SEs (Anton et al., 2010). Due to the solid  
591 nature of the preload, it is possible that the satiating impact of the S&SEs in that study may have  
592 been enhanced vs a liquid preload, either via the texture or other food characteristics (Almiron-  
593 Roig et al., 2013; Appleton et al., 2021). In line with our results, another study (Tey et al., 2017b),  
594 also failed to detect an impact in total energy intakes after a sucrose or stevia RebA beverage  
595 preload.

596 Contrary to preload beverages containing mogroside alone (Tey et al., 2017b), mogroside  
597 together with steviol (RebM) in our study did not induce higher appetite ratings, confirmed by  
598 comparable total energy intakes vs the sucrose condition. Our findings are still relevant as the  
599 dosage of both mogroside and sucrose used in the Tey's study were higher [0.63 g mogroside  
600 extract exclusively in Tey's vs 0.13 g in this study (as blend); and 65 g sucrose in Tey's vs 26 g in  
601 the present study] (Tey et al., 2017b). Therefore, the impact on glycaemic and appetite responses  
602 are still visible at these much lower concentrations.

603 A beverage containing ace-K with sucralose with and without aspartame marginally increased  
604 insulin AUCs (by 22- 25 %) and glucose-induced GLP-1 secretion in a previous study without  
605 impacting on glycaemia (Sylvetsky et al., 2016). Ace-K, but not sucralose, alone was also found to  
606 exert a small impact on glycaemia when administered in doses equivalent to habitual  
607 consumption (Bryant et al., 2014). Ace-K differs from other S&SEs because it activates bitter taste  
608 receptors at lower concentrations (Dotson et al., 2008). Overall, the impact of sucralose on  
609 glycaemic response is under debate (Grotz & Jokinen, 2014; Khan & Sievenpiper, 2021; Pepino et  
610 al., 2013; Sylvetsky et al., 2016; Yunker et al., 2021) and it is unknown by which mechanism  
611 sucralose's effects, if real, happen (e.g. by activation of pancreatic or intestinal sweet taste  
612 receptors) (Buchanan et al., 2022; Sylvetsky & Rother, 2018). Some of these studies have used  
613 beverages containing other ingredients (i.e. cola-based, caffeine-free sodas), which may have  
614 confounded the results.

615 Concerning the appetite response, our findings support the concept that some S&SEs induce  
616 higher subjective appetite and lower subjective fullness compared with caloric controls (Tey et  
617 al., 2017b). However, we detected no changes in the ni-AUC values for the appetite VAS and no  
618 impacts on total energy intakes. While some studies have found related S&SE blends to impact  
619 similarly on appetite (e.g. containing sucralose) (Sylvetsky et al., 2016), stevia and aspartame  
620 preloads were equally satiating in other studies (Anton et al., 2010; Stamataki, Scott, et al., 2020).  
621 In the present work, both blends with steviol glycosides seemed to control appetite better  
622 compared with the sucralose blend irrespective of time course, therefore the potential different  
623 mechanisms of action are worthy of further investigation.

624 The Suc\_Ace beverage was similarly liked and desired as the energy-containing sucrose control,  
625 while the novel stevia blends had a lower acceptance, although still close to 60%. Unpublished  
626 data suggest that the Suc\_Ace blend was also associated with a lower craving control compared  
627 with all other conditions (that is, regardless of energy content). It has been suggested that  
628 sucralose can increase reward responses to specific food cues in women and in persons living  
629 with obesity (Yunker et al., 2021), which would initially agree with our observations. It is also  
630 known that S&SE can bind to different regions of the sweet taste receptor heterodimer (Kim et

631 al., 2017) and in gut sensor cells T13R receptors (Buchanan et al., 2022) unchaining distinct  
632 patterns of intracellular signals which likely contribute to each S&SE sensory profile, pre-ingestive  
633 responses and downstream effects (Higgins & Mattes, 2019).

634

#### 635 **4.1. Strengths and limitations**

636 This study overcomes a number of limitations identified in a previous systematic review on the  
637 impact of S&SE on the glycaemic response (Greylling et al., 2020). First, this was a large cross-  
638 European trial involving blends, as opposed to single sweeteners, which allowed for an increase  
639 in sweetness and reduction in off-tastes (Feder, 2012; Michail, 2017; Pawar et al., 2013). As a  
640 result, smaller doses of some S&SEs were used compared with some previous studies using single  
641 doses. Our findings on the metabolic impact in particular for the stevia blends and for thaumatin  
642 (lacking published clinical data), may be useful as part of any ongoing assessments of these  
643 S&SEs. The cross-European nature of the trial may help to generalise the results among habitual  
644 S&SE consumers.

645 Second, the present study used of a tightly controlled cross-over design with exclusion of normal-  
646 weight participants, which ensured a relatively low inter-individual variability. The study was also  
647 double-blinded, which is often not possible in nutritional interventions.

648 The wide range of endpoints assessed was made feasible by the multidisciplinary approach of  
649 this work and the relatively large sample size, compared with similar studies. This was particularly  
650 useful in the analysis of covariates for appetite ratings and metabolic markers, allowing detection  
651 of subtler differences between S&SE blends, beyond the control condition. Also, treatment order  
652 effects were minimal and did not modulate the impact of blend on appetite ratings.

653 The intervention beverages were delivered very close in time with a standardised breakfast  
654 providing about 1/3 of the individual's daily requirements, including 50-80 g of carbohydrate,  
655 which likely attenuated the impact of the blends on blood glucose levels and later energy intakes.  
656 A different type of meal (e.g. fat or protein-rich) may have induced different glycaemic and lipid  
657 responses and could also be affected by the nutritional status of the participants (Movahedian et

658 al., 2021). This design was purposely chosen to simulate normal eating and drinking situations  
659 and to maximize the impact of the intervention product providing virtually no energy (for which  
660 little or no compensatory behaviour was expected) (Almiron-Roig et al., 2013). Although, such a  
661 design makes the interpretation of the true effects of each blend more difficult, the purpose was  
662 to see if blends given before a fixed meal resulted in different glycaemic responses, and this was  
663 achieved. A retrospective power calculation taking into account multiple comparisons estimated  
664 the reached power to be 50-82% for the glucose iAUCs comparisons vs. sucrose, and of 90-92%  
665 for the insulin iAUC comparisons.

666 The beverages were designed to be matched for sweetness intensity, bitterness intensity and  
667 other sensory characteristics. Although, sensory analyses failed to show total similarity in  
668 sweetness levels in each of the beverages, differences in liking and desire were moderate and  
669 did not significantly affect appetite and energy intakes except for minor changes in fullness and  
670 desire to eat ratings. As this was an acute postprandial study, the effects of longer and larger  
671 doses of S&SEs were not analysed. These effects may differ depending on trial duration and  
672 repeated exposure. Finally, participant-specific, individual responses were not fully investigated.  
673 For example, insulin sensitivity may be affected by menstrual cycle (Grotz & Jokinen, 2014) and  
674 this was not controlled for. However, exposure conditions were randomised and the trial lasted  
675 for approximately 4-6 weeks, which hopefully helped counterbalancing the potential effect of  
676 menstrual cycle.

677

## 678 **4.2. Conclusions**

679 The results of this investigation confirm the neutral or beneficial impact in acute glycaemic  
680 control arising from combining plant-based S&SEs such as stevia RebA, stevia RebM, thaumatin,  
681 and mogroside V, compared with a sucrose-yielding beverage. The explored S&SEs in beverage  
682 format could be used to improve the glycaemic response to a meal without significant negative  
683 effects on acute food intake behaviour or body metabolism, which would support their potential

684 role in the prevention and management of diabetes and for body weight management, as part  
685 of a wider lifestyle approach.

686

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696

### 697 **5.2. Author's contributions**

698 JAH, JCGH, and ARA are the SWEET project coordinators. JAM and GF are leader and co-leader  
699 for this acute clinical trial work package in SWEET; CS coordinated the S&SEs selection process,  
700 design and manufacturing of the beverages and sensory analyses. MMR led the Consumers'  
701 Perspectives theme and implemented the Qualtrics survey alongside CEH. EAR, LK, and CAH led  
702 the intervention studies at Navarra, Copenhagen, and Liverpool respectively, with support from  
703 SNC, JAM, AR, and JAH. GC, MN, LK, and NM led the data collection. AR is the datahub manager  
704 for the acute trials and performed the data analysis for all sites. HM performed the biochemical  
705 analyses. EAR wrote the first draft of the paper. All authors provided revisions and have approved  
706 the final version of the manuscript.

707

708



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710 SWEET has received funding from the EU's Horizon 2020 research & innovation programme  
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### 712 **5.4. Data and code availability**

713 Anonymized data for this study will be uploaded onto an open source repository (UK Data  
714 Archive) by Jan 2028. Programming codes for the QDP are available from the corresponding  
715 author upon request via a formal data sharing agreement.

### 716 **5.5. Declaration of competing interest**

717 JCGH, JAH, and CAH and are in receipt of research funding from the American Beverage  
718 Association; MMR and CEH's research centre provides consultancy to, and has received travel  
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721 CAH has received honoraria from the International Sweeteners Association. CS is an employee of  
722 Cargill, Inc. The other authors have nothing to declare.

723

### 724 **REFERENCES**

725 Almiron-Roig, E, Green, H., Virgili, R., Aeschlimann, J. M., Moser, M., & Erkner, A. (2009). Validation of a  
726 new hand-held electronic appetite rating system against the pen and paper method. *Appetite*,  
727 53(3), 465–468. [https://doi.org/S0195-6663\(09\)00630-8](https://doi.org/S0195-6663(09)00630-8) [pii] 10.1016/j.appet.2009.09.014

728 Almiron-Roig, Eva, & Drewnowski, A. (2003). Hunger, thirst, and energy intakes following consumption  
729 of caloric beverages. *Physiology and Behavior*, 79(4–5), 767–773. [https://doi.org/10.1016/S0031-9384\(03\)00212-9](https://doi.org/10.1016/S0031-9384(03)00212-9)

731 Almiron-Roig, Eva, Palla, L., Guest, K., Ricchiuti, C., Vint, N., Jebb, S. A., & Drewnowski, A. (2013). Factors  
732 that determine energy compensation: a systematic review of preload studies. *Nutrition Reviews*,  
733 71(7), 458–473. <https://doi.org/10.1111/nure.12048>

734 American Diabetes Association. (2008). Standards of medical care in diabetes - 2008. In *Diabetes Care*  
735 (Vol. 31, Issue SUPPL. 1, pp. S12-54). Diabetes Care. <https://doi.org/10.2337/dc08-S012>

736 Anton, S. D., Martin, C. K., Han, H., Coulon, S., Cefalu, W. T., Geiselman, P., & Williamson, D. A. (2010).

- 737 Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and  
738 insulin levels. *Appetite*, 55(1), 37–43. <https://doi.org/10.1016/j.appet.2010.03.009>
- 739 Appleton, K. M., Newbury, A., Almiron-Roig, E., Yeomans, M. R., Brunstrom, J. M., de Graaf, K., Geurts,  
740 L., Kildegaard, H., & Vinoy, S. (2021). Sensory and physical characteristics of foods that impact food  
741 intake without affecting acceptability: Systematic review and meta-analyses. *Obesity Reviews*,  
742 22(8), e13234. <https://doi.org/10.1111/obr.13234>
- 743 Ascaso, J. F., Real, J. T., Priego, A., Carmena, R., Romero, P., & Valdecabres, C. (2001). Insulin resistance  
744 quantification by fasting insulin plasma values and HOMA index in a non-diabetic population.  
745 *Medicina Clinica*, 117(14), 530–533. [https://doi.org/10.1016/S0025-7753\(01\)72168-9](https://doi.org/10.1016/S0025-7753(01)72168-9)
- 746 Australian Bureau of Statistics. (2010). *Australian Health Survey: Users' Guide; 2011-13*. 4363.0.55.001.  
747 Australian Health Survey Food Model Booklet; c=AU; o=Commonwealth of Australia; ou=Australian  
748 Bureau of Statistics.  
749 <https://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/4363.0.55.0012011-13?OpenDocument>
- 750 Bedogni, G., Bellentani, S., Miglioli, L., Masutti, F., Passalacqua, M., Castiglione, A., & Tiribelli, C. (2006).  
751 The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population.  
752 *BMC Gastroenterology*, 6(1), 33. <https://doi.org/10.1186/1471-230X-6-33>
- 753 Booth, M. (2000). Assessment of physical activity: An international perspective. *Research Quarterly for*  
754 *Exercise and Sport*, 71(2 Suppl), 114–120. <https://doi.org/10.1080/02701367.2000.11082794>
- 755 Bradley, R., Kozura, E., Buckle, H., Kaltunas, J., Tais, S., & Standish, L. J. (2009). Description of clinical risk  
756 factor changes during naturopathic care for type 2 diabetes. *Journal of Alternative and*  
757 *Complementary Medicine (New York, N.Y.)*, 15(6), 633–638.  
758 <https://doi.org/10.1089/acm.2008.0249>
- 759 Brandt, K. R., Sünram-Lea, S. I., & Qualtrough, K. (2006). The effect of glucose administration on the  
760 emotional enhancement effect in recognition memory. *Biological Psychology*, 73(2), 199–208.  
761 <https://doi.org/10.1016/j.biopsycho.2006.04.001>
- 762 British Dietetic Association. (2016). *The use of artificial sweeteners*. Policy Statement.  
763 [https://www.bda.uk.com/uploads/assets/11ea5867-96eb-43df-](https://www.bda.uk.com/uploads/assets/11ea5867-96eb-43df-b61f2cbe9673530d/policystatementsweetners.pdf)  
764 [b61f2cbe9673530d/policystatementsweetners.pdf](https://www.bda.uk.com/uploads/assets/11ea5867-96eb-43df-b61f2cbe9673530d/policystatementsweetners.pdf)
- 765 Brouns, F., Bjorck, I., Frayn, K. N., Gibbs, A. L., Lang, V., Slama, G., & Wolever, T. M. S. (2005). Glycaemic  
766 index methodology. *Nutrition Research Reviews*, 18(1), 145–171.  
767 <https://doi.org/10.1079/nrr2005100>
- 768 Bryant, C. E., Wasse, L. K., Astbury, N., Nandra, G., & McLaughlin, J. T. (2014). Non-nutritive sweeteners:  
769 No class effect on the glycaemic or appetite responses to ingested glucose. *European Journal of*  
770 *Clinical Nutrition*, 68(5), 629–631. <https://doi.org/10.1038/ejcn.2014.19>
- 771 Buchanan, K. L., Rupprecht, L. E., Kaelberer, M. M., Sahasrabudhe, A., Klein, M. E., Villalobos, J. A., Liu,  
772 W. W., Yang, A., Gelman, J., Park, S., Anikeeva, P., & Bohórquez, D. V. (2022). The preference for  
773 sugar over sweetener depends on a gut sensor cell. *Nature Neuroscience*, 25(2), 191–200.  
774 <https://doi.org/10.1038/s41593-021-00982-7>

- 775 Centers for Disease Control and Prevention. (n.d.). *National Health and Nutrition Examination Survey*.  
776 Retrieved March 30, 2017, from <https://www.cdc.gov/nchs/nhanes/>
- 777 Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. L. Erlbaum Associates.  
778 [https://books.google.es/books?id=2v9zDAsLvA0C&pg=PP1&redir\\_esc=y#v=onepage&q&f=false](https://books.google.es/books?id=2v9zDAsLvA0C&pg=PP1&redir_esc=y#v=onepage&q&f=false)
- 779 Dalenberg, J. R., Patel, B. P., Denis, R., Veldhuizen, M. G., Nakamura, Y., Vinke, P. C., Luquet, S., & Small,  
780 D. M. (2020). Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs  
781 Neural and Metabolic Sensitivity to Sugar in Humans. *Cell Metabolism*, 31(3), 493-502.e7.  
782 <https://doi.org/10.1016/j.cmet.2020.01.014>
- 783 Dotson, C. D., Zhang, L., Xu, H., Shin, Y. K., Vignes, S., Ott, S. H., Elson, A. E. T., Choi, H. J., Shaw, H., Egan,  
784 J. M., Mitchell, B. D., Li, X., Steinle, N. I., & Munger, S. D. (2008). Bitter taste receptors influence  
785 glucose homeostasis. *PLoS ONE*, 3(12). <https://doi.org/10.1371/journal.pone.0003974>
- 786 Douglas, S. M., & Leidy, H. J. (2019). Novel Methodological Considerations Regarding the Use of Visual  
787 Analog Scale (VAS) Appetite Questionnaires in Tightly Controlled Feeding Trials. *Current*  
788 *Developments in Nutrition*, 3(6), nzz061. <https://doi.org/10.1093/CDN/NZZ061>
- 789 Ebbeling, C. B., Feldman, H. A., Chomitz, V. R., Antonelli, T. A., Gortmaker, S. L., Osganian, S. K., &  
790 Ludwig, D. S. (2012). A Randomized Trial of Sugar-Sweetened Beverages and Adolescent Body  
791 Weight. *New England Journal of Medicine*, 367(15), 1407–1416.  
792 <https://doi.org/10.1056/NEJMoa1203388>
- 793 EFSA. (2011). EFSA | European Food Safety Authority. Scientific Opinion on the substantiation of health  
794 claims related to intense sweeteners and contribution to the maintenance or achievement of a  
795 normal body weight (ID 1136, 1444, 4299), reduction of post-prandial gly. *EFSA Journal*, 9(6).  
796 <https://doi.org/10.2903/j.efsa.2011.2229>
- 797 Feder, D. (2012). *How Sweet it is*. Prepared Foods. [https://www.preparedfoods.com/articles/111643-](https://www.preparedfoods.com/articles/111643-how-sweet-it-is-)  
798 [how-sweet-it-is-](https://www.preparedfoods.com/articles/111643-how-sweet-it-is-)
- 799 Finlayson, G., King, N., & Blundell, J. (2008). The role of implicit wanting in relation to explicit liking and  
800 wanting for food: Implications for appetite control. *Appetite*, 50(1), 120–127.  
801 <https://doi.org/10.1016/j.appet.2007.06.007>
- 802 Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual  
803 analogue scales in assessment of appetite sensations in single test meal studies. *International*  
804 *Journal of Obesity and Related Metabolic Disorders*, 24(1), 38–48.  
805 <http://www.ncbi.nlm.nih.gov/pubmed/10702749>
- 806 Forestfield Software Ltd. (2021). *Dietplan7*. On-Line Software. <http://dietplan7.com/index.html>
- 807 Foster, E., Hawkins, A., Barton, K. L., Stamp, E., Matthews, J. N. S., & Adamson, A. J. (2017). Development  
808 of food photographs for use with children aged 18 months to 16 years: Comparison against  
809 weighed food diaries – The Young Person’s Food Atlas (UK). *PLOS ONE*, 12(2), e0169084.  
810 <https://doi.org/10.1371/journal.pone.0169084>
- 811 Gallagher, A. M., Ashwell, M., Halford, J. C. G., Hardman, C. A., Maloney, N. G., & Raben, A. (2021). Low-  
812 calorie sweeteners in the human diet: Scientific evidence, recommendations, challenges and future

- 813 needs. A symposium report from the FENS 2019 conference. In *Journal of Nutritional Science* (Vol.  
814 10, p. e7). *J Nutr Sci*. <https://doi.org/10.1017/jns.2020.59>
- 815 Garner, D. M., & Garfinkel, P. E. (1979). The Eating Attitudes Test: an index of the symptoms of anorexia  
816 nervosa. *Psychological Medicine*, *9*(2), 273–279. <http://www.ncbi.nlm.nih.gov/pubmed/472072>
- 817 Gayoso-Diz, P., Otero-González, A., Rodríguez-Alvarez, M. X., Gude, F., García, F., De Francisco, A., &  
818 Quintela, A. G. (2013). Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a  
819 general adult population: Effect of gender and age: EPIRCE cross-sectional study. *BMC Endocrine*  
820 *Disorders*, *13*, 47. <https://doi.org/10.1186/1472-6823-13-47>
- 821 Graaf, C. De, & Frijters, J. E. R. (1986). A psychophysical investigation of Beidler’s mixture equation.  
822 *Chemical Senses*, *11*(3), 295–314. <https://doi.org/10.1093/chemse/11.3.295>
- 823 Green, M. W., Taylor, M. A., Elliman, N. A., & Rhodes, O. (2001). Placebo expectancy effects in the  
824 relationship between glucose and cognition. *British Journal of Nutrition*, *86*(2), 173–179.  
825 <https://doi.org/10.1079/bjn2001398>
- 826 Greylling, A., Appleton, K. M., Raben, A., & Mela, D. J. (2020). Acute glycaemic and insulinemic effects of  
827 low-energy sweeteners: a systematic review and meta-analysis of randomized controlled trials. *Am*  
828 *J Clin Nutr*, *112*(4), 1002–1014. <https://doi.org/doi:10.1093/ajcn/nqaa167>
- 829 Grotz, V. L., & Jokinen, J. D. (2014). Sucralose affects glycaemic and hormonal responses to an oral  
830 glucose load. In *Diabetes Care* (Vol. 37, Issue 6, pp. e148–e148). American Diabetes Association.  
831 <https://doi.org/10.2337/dc13-2972>
- 832 Healthcare Software Solutions S.A. (2021). *Nutrium | All-in-one Nutrition Software: Nutrition Analysis &*  
833 *CRM*. On-Line Software. <https://nutrium.com/en>
- 834 Higgins, K. A., & Mattes, R. D. (2019). A randomized controlled trial contrasting the effects of 4 low-  
835 calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *American*  
836 *Journal of Clinical Nutrition*, *109*(5), 1288–1301. <https://doi.org/10.1093/ajcn/nqy381>
- 837 Hill, A. J., & Blundell, J. E. (1982). Nutrients and behaviour: Research strategies for the investigation of  
838 taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of*  
839 *Psychiatric Research*, *17*(2), 203–212. [https://doi.org/10.1016/0022-3956\(82\)90023-1](https://doi.org/10.1016/0022-3956(82)90023-1)
- 840 Jiménez-Domínguez, G., Ble-Castillo, J. L., Aparicio-Trápala, M. A., Juárez-Rojop, I. E., Tovilla-Zárate, C. A.,  
841 Ble-Castillo, D. J., García-Vázquez, C., Olvera-Hernández, V., Pérez-Pimienta, B., Díaz-Zagoya, J. C.,  
842 & Mendez, J. D. (2015). Effects of acute ingestion of native banana starch on glycaemic response  
843 evaluated by continuous glucose monitoring in obese and lean subjects. *International Journal of*  
844 *Environmental Research and Public Health*, *12*(7), 7491–7505.  
845 <https://doi.org/10.3390/ijerph120707491>
- 846 Jones, B., & Kenward, M. G. (2015). *Design and Analysis of Cross-Over Trials* (3rd ed.). Chapman and  
847 Hall/ CRC. <https://www.amazon.com/Analysis-Cross-Over-Monographs-Statistics-Probability/dp/1439861420>
- 849 Kassambara, A. (2022, November 9). *Pipe-Friendly Framework for Basic Statistical Tests [R package*  
850 *rstatix version 0.7.1]*. Comprehensive R Archive Network (CRAN). <https://cran.r->

- 851 project.org/package=rstatix
- 852 Khan, T. A., & Sievenpiper, J. L. (2021). Low-Calorie Sweeteners with Carbohydrate Do Not Impair Insulin  
853 Sensitivity in Humans: Re-analysis Highlighting the Importance of the Comparator. *Cell Metabolism*,  
854 33(2), 225–226. <https://doi.org/10.1016/J.CMET.2020.10.024>
- 855 Kim, S. K., Chen, Y., Abrol, R., Goddard, W. A., & Guthrie, B. (2017). Activation mechanism of the G  
856 protein-coupled sweet receptor heterodimer with sweeteners and allosteric agonists. *Proceedings*  
857 *of the National Academy of Sciences of the United States of America*, 114(10), 2568–2573.  
858 <https://doi.org/10.1073/pnas.1700001114>
- 859 Kraftaerk Foodtech. (n.d.). *Dankost Pro er anerkendt af hele fødevarerbranchen - læs mere her*. On-Line  
860 Software. Retrieved September 22, 2021, from <https://www.kraftvaerk-foodtech.com/da>
- 861 Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical  
862 primer for t-tests and ANOVAs. *Frontiers in Psychology*, 4(NOV), 863.  
863 <https://doi.org/10.3389/fpsyg.2013.00863>
- 864 Lee, H. Y., Jack, M., Poon, T., Noori, D., Venditti, C., Hamamji, S., & Musa-Veloso, K. (2021). Effects of  
865 Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners on  
866 Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled Human  
867 Intervention Studies. In *Advances in Nutrition* (Vol. 12, Issue 4, pp. 1481–1499). Adv Nutr.  
868 <https://doi.org/10.1093/advances/nmaa157>
- 869 Malik, V. S., & Hu, F. B. (2022). The role of sugar-sweetened beverages in the global epidemics of obesity  
870 and chronic diseases. In *Nature Reviews Endocrinology* (Vol. 18, Issue 4, pp. 205–218). Nat Rev  
871 Endocrinol. <https://doi.org/10.1038/s41574-021-00627-6>
- 872 Michail, N. (2017). *Monk fruit-stevia blends are still best for natural sugar reduction, says Layn*. Food  
873 Navigator,. [https://www.foodnavigator.com/Article/2017/12/05/Monk-fruit-stevia-blends-are-](https://www.foodnavigator.com/Article/2017/12/05/Monk-fruit-stevia-blends-are-still-best-for-natural-sugar-reduction-says-Layn#)  
874 [still-best-for-natural-sugar-reduction-says-Layn#](https://www.foodnavigator.com/Article/2017/12/05/Monk-fruit-stevia-blends-are-still-best-for-natural-sugar-reduction-says-Layn#)
- 875 Mora, M. R., & Dando, R. (2021). The sensory properties and metabolic impact of natural and synthetic  
876 sweeteners. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 20, Issue 2, pp. 1554–  
877 1583). John Wiley & Sons, Ltd. <https://doi.org/10.1111/1541-4337.12703>
- 878 Movahedian, M., Golzan, S. A., Ashtary-Larky, D., Clark, C. C. T., Asbaghi, O., & Hekmatdoost, A. (2021).  
879 The effects of artificial- and stevia-based sweeteners on lipid profile in adults: a GRADE-assessed  
880 systematic review, meta-analysis, and meta-regression of randomized clinical trials. In *Critical*  
881 *Reviews in Food Science and Nutrition* (pp. 1–17). Crit Rev Food Sci Nutr.  
882 <https://doi.org/10.1080/10408398.2021.2012641>
- 883 Nichol, A. D., Holle, M. J., & An, R. (2018). Glycemic impact of non-nutritive sweeteners: A systematic  
884 review and meta-Analysis of randomized controlled trials. In *European Journal of Clinical Nutrition*  
885 (Vol. 72, Issue 6, pp. 796–804). Eur J Clin Nutr. <https://doi.org/10.1038/s41430-018-0170-6>
- 886 Nordic Council of Ministers. (n.d.). *Nordic nutrition recommendations 2012 : integrating nutrition and*  
887 *physical activity*.
- 888 O'Connor, D., Pang, M., Castelnuovo, G., Finlayson, G., Blaak, E., Gibbons, C., Navas-Carretero, S.,

- 889 Almiron-Roig, E., Harrold, J., Raben, A., & Martinez, J. A. (2021). A rational review on the effects of  
890 sweeteners and sweetness enhancers on appetite, food reward and metabolic/adiposity outcomes  
891 in adults. In *Food and Function* (Vol. 12, Issue 2, pp. 442–465).  
892 <https://doi.org/10.1039/d0fo02424d>
- 893 Pawar, R. S., Krynitsky, A. J., & Rader, J. I. (2013). Sweeteners from plants-with emphasis on Stevia  
894 rebaudiana (Bertoni) and Siraitia grosvenorii (Swingle). In *Analytical and Bioanalytical Chemistry*  
895 (Vol. 405, Issue 13, pp. 4397–4407). Springer Verlag. <https://doi.org/10.1007/s00216-012-6693-0>
- 896 Pepino, M. Y., Tiemann, C. D., Patterson, B. W., Wice, B. M., & Klein, S. (2013). Sucralose affects glycemic  
897 and hormonal responses to an oral glucose load. *Diabetes Care*, *36*(9), 2530–2535.  
898 <https://doi.org/10.2337/dc12-2221>
- 899 Public Health England. (2020). *NDNS: results from years 9 to 11 (2016 to 2017 and 2018 to 2019)*.  
900 National Diet and Nutrition Survey. [https://www.gov.uk/government/statistics/ndns-results-from-](https://www.gov.uk/government/statistics/ndns-results-from-years-9-to-11-2016-to-2017-and-2018-to-2019)  
901 [years-9-to-11-2016-to-2017-and-2018-to-2019](https://www.gov.uk/government/statistics/ndns-results-from-years-9-to-11-2016-to-2017-and-2018-to-2019)
- 902 Rios-Leyvraz, M., & Montez, J. (2022). *Health effects of the use of non-sugar sweeteners: a systematic*  
903 *review and meta-analysis*. Geneva: World Health Organization. World Health Organization.,  
904 <https://www.who.int/publications/i/item/9789240046429>
- 905 Robinson, E., Nolan, S., Tudur-Smith, C., Boyland, E. J., Harrold, J. A., Hardman, C. A., & Halford, J. C.  
906 (2014). Will smaller plates lead to smaller waists? A systematic review and meta-analysis of the  
907 effect that experimental manipulation of dishware size has on energy consumption. *Obes Rev*,  
908 *15*(10), 812–821. <https://doi.org/10.1111/obr.12200>
- 909 Rogers, P J, Hogenkamp, P. S., de Graaf, C., Higgs, S., Lluch, A., Ness, A. R., Penfold, C., Perry, R., Putz, P.,  
910 Yeomans, M. R., & Mela, D. J. (2016). Does low-energy sweetener consumption affect energy  
911 intake and body weight? A systematic review, including meta-analyses, of the evidence from  
912 human and animal studies. *International Journal of Obesity (2005)*, *40*(3), 381–394.  
913 <https://doi.org/10.1038/ijo.2015.177>
- 914 Rogers, Peter J., & Hardman, C. A. (2015). Food reward. What it is and how to measure it. *Appetite*, *90*,  
915 1–15. <https://doi.org/10.1016/j.appet.2015.02.032>
- 916 Ruiz, E., Rodriguez, P., Valero, T., Ávila, J. M., Aranceta-Bartrina, J., Ángel, G., González-Gross, M.,  
917 Ortega, R. M., Serra-majem, L., & Varela-moreiras, G. (2017). Dietary Intake of Individual ( Free and  
918 Intrinsic ) Sugars and Food Sources in the Spanish Population : Findings from the ANIBES Study.  
919 *Nutrients*, *9*(275). <https://doi.org/10.3390/nu9030275>
- 920 Schiffman, S. S., Sattely-Miller, E. A., Graham, B. G., Zervakis, J., Butchko, H. H., & Stargel, W. W. (2003).  
921 Effect of repeated presentation on sweetness intensity of binary and ternary mixtures of  
922 sweeteners. *Chemical Senses*, *28*(3), 219–229. <https://doi.org/10.1093/chemse/28.3.219>
- 923 Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting  
924 glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy  
925 subjects. *Metabolic Syndrome and Related Disorders*, *6*(4), 299–304.  
926 <https://doi.org/10.1089/met.2008.0034>
- 927 Singh, G. M., Micha, R., Khatibzadeh, S., Shi, P., Lim, S., Andrews, K. G., Engell, R. E., Ezzati, M.,

- 928 Mozaffarian, D., Fahimi, S., Powles, J., Elmadfa, I., Rao, M., Wirojratana, P., Abbott, P. A., Abdollahi,  
929 M., Gilardon, E. A., Ahsan, H., Al Nsour, M. A. A., ... Zajkás, G. (2015). Global, regional, and national  
930 consumption of sugar-sweetened beverages, fruit juices, and milk: A systematic assessment of  
931 beverage intake in 187 countries. *PLoS ONE*, *10*(8), e0124845.  
932 <https://doi.org/10.1371/journal.pone.0124845>
- 933 Stamataki, N. S., Crooks, B., Ahmed, A., & McLaughlin, J. T. (2020). Effects of the daily consumption of  
934 stevia on glucose homeostasis, body weight, and energy intake: A randomised open-label 12-week  
935 trial in healthy adults. *Nutrients*, *12*(10), 1–16. <https://doi.org/10.3390/nu12103049>
- 936 Stamataki, N. S., Scott, C., Elliott, R., McKie, S., Bosscher, D., & McLaughlin, J. T. (2020). Stevia beverage  
937 consumption prior to lunch reduces appetite and total energy intake without affecting glycemia or  
938 attentional bias to food cues: A double-Blind randomized controlled trial in healthy adults. *Journal*  
939 *of Nutrition*, *150*(5), 1126–1134. <https://doi.org/10.1093/jn/nxaa038>
- 940 Suez, J., Korem, T., Zeevi, D., Zilberman-Schapira, G., Thaiss, C. A., Maza, O., Israeli, D., Zmora, N., Gilad,  
941 S., Weinberger, A., Kuperman, Y., Harmelin, A., Kolodkin-Gal, I., Shapiro, H., Halpern, Z., Segal, E., &  
942 Elinav, E. (2014). Artificial sweeteners induce glucose intolerance by altering the gut microbiota.  
943 *Nature*, *514*(7521), 181–186. <https://doi.org/10.1038/nature13793>
- 944 Svedlund, J., Sjodin, I., & Dotevall, G. (1988). GSRS-A clinical rating scale for gastrointestinal symptoms in  
945 patients with irritable bowel syndrome and peptic ulcer disease. *Digestive Diseases and Sciences*,  
946 *33*(2), 129–134. <https://doi.org/10.1007/BF01535722>
- 947 SWEET Project. (2019). *SWEET - Sweeteners and sweetness enhancers: Impact on health, obesity, safety*  
948 *and sustainability (774293)*. <https://sweetproject.eu/>
- 949 Sylvetsky, A. C., Brown, R. J., Blau, J. E., Walter, M., & Rother, K. I. (2016). Hormonal responses to non-  
950 nutritive sweeteners in water and diet soda. *Nutrition and Metabolism*, *13*(1), 1–8.  
951 <https://doi.org/10.1186/s12986-016-0129-3>
- 952 Sylvetsky, A. C., & Rother, K. I. (2018). Nonnutritive Sweeteners in Weight Management and Chronic  
953 Disease: A Review. In *Obesity* (Vol. 26, Issue 4, pp. 635–640). NIH Public Access.  
954 <https://doi.org/10.1002/oby.22139>
- 955 Tey, S. L., Salleh, N. B., Henry, C. J., & Forde, C. G. (2017a). Effects of non-nutritive (artificial vs natural)  
956 sweeteners on 24-h glucose profiles. *European Journal of Clinical Nutrition*, *71*(9), 1129–1132.  
957 <https://doi.org/10.1038/ejcn.2017.37>
- 958 Tey, S. L., Salleh, N. B., Henry, J., & Forde, C. G. (2017b). Effects of aspartame-, monk fruit-, stevia- and  
959 sucrose-sweetened beverages on postprandial glucose, insulin and energy intake. *International*  
960 *Journal of Obesity*, *41*(3), 450–457. <https://doi.org/10.1038/ijo.2016.225>
- 961 Tjønneland, A., Olsen, A., Boll, K., Stripp, C., Christensen, J., Engholm, G., & Overvad, K. (2007). Study  
962 design, exposure variables, and socioeconomic determinants of participation in Diet, Cancer and  
963 Health: A population-based prospective cohort study of 57,053 men and women in Denmark.  
964 *Scandinavian Journal of Public Health*, *35*(4), 432–441.  
965 <https://doi.org/10.1080/14034940601047986>
- 966 Tucker, R. M., & Tan, S. Y. (2017). Do non-nutritive sweeteners influence acute glucose homeostasis in

- 967 humans? A systematic review. In *Physiology and Behavior* (Vol. 182, pp. 17–26). Elsevier Inc.  
968 <https://doi.org/10.1016/j.physbeh.2017.09.016>
- 969 WHO. (2015). *WHO | Guideline: Sugars intake for adults and children*. World Health Organization.  
970 <https://www.who.int/publications/i/item/9789241549028>
- 971 WHO. (2018). *WHO | Noncommunicable diseases country profiles 2018*. World Health Organization;  
972 World Health Organization. <https://doi.org/10.3109/21691401.2015.1058807>
- 973 Wolever, T. M., Jenkins, D. J., Jenkins, A. L., & Josse, R. G. (1991). The glycemic index: methodology and  
974 clinical implications. *The American Journal of Clinical Nutrition*, 54(5), 846–854.  
975 <https://doi.org/10.1093/ajcn/54.5.846>
- 976 Yunker, A. G., Alves, J. M., Luo, S., Angelo, B., DeFendis, A., Pickering, T. A., Monterosso, J. R., & Page, K.  
977 A. (2021). Obesity and Sex-Related Associations With Differential Effects of Sucralose vs Sucrose on  
978 Appetite and Reward Processing: A Randomized Crossover Trial. *JAMA Network Open*, 4(9),  
979 e2126313–e2126313. <https://doi.org/10.1001/JAMANETWORKOPEN.2021.26313>
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## Supplementary Material

**Impact of acute consumption of beverages containing plant-based or alternative sweetener blends on postprandial appetite, food intake, metabolism, and gastro-intestinal symptoms: results of the SWEET Beverages trial**

Almiron-Roig, Navas-Carretero, Castelnuovo *et al.*

(*Appetite*)

### **Methods**

#### **Exclusion criteria for participants**

- blood donation within the last 3 months, malnutrition or dehydration
- food allergy, intolerance, restriction, or avoidance of any of the study foods
- likelihood for disordered eating defined as a score of 20 or more on the Eating Attitudes Test (EAT)-26 [1]
- currently dieting to lose weight or having been on weight cycles in the last 3 months
- smoking (or <2 months since quitting)
- binge drinking
- performing >10 h of intense physical activity per week
- continuous night or late shift work; self-reported use of drugs of abuse within the previous 12 months
- for women, pregnancy or lactation
- taking medication for or a history of medical conditions affecting body weight, appetite, and G.I. function e.g. diabetes mellitus, inflammatory bowel diseases, surgical treatment of obesity, history of cancer, cardio-vascular disease, cirrhosis, unstable thyroid disease, and psychiatric illness. Low-dose antidepressants, cholesterol-lowering medication, and treatment for hypothyroidism were allowed if the person had been on a stable dose for at least 3 months
- not having access to either (mobile) phone or internet
- insufficient communication in the national language
- suspected to be unable to follow the study protocol
- with previous university or college training related to eating behaviour research.

1015 **Pre-study sensory analysis**

1016 Initial sensory analyses by Cargill confirmed reasonable acceptance for all intervention  
1017 beverages. Overall liking was between 4.4 and 6.1 points on a 9-point hedonic scale, despite  
1018 moderate differences in sweet taste perception (range 47-67 out of 100 mm).

1019

1020

1021 **Additional questionnaires information**

1022

1023 *Habitual consumption of sweet foods* (both regularly sweetened and sweetened with artificial  
1024 sweeteners) was evaluated with a self-constructed, short sweet food frequency questionnaire  
1025 (sFFQ), developed based on previous work [2]. This included a list of 11 items representing the  
1026 most important sources of sugar e.g., 80% of all sources, adapted for each country.

1027

1028 *Physical activity* was measured with the IPAQ long form [3].

1029

1030 *Socio-demographic characteristics and perceptions of the intervention* (end of study survey in Fig.  
1031 1) were collected using self-constructed questionnaires based on previous work [4,5]. Both are  
1032 available upon request.

1033

1034 *Consumer perceptions of S&SEs* (Consumer Perspectives Survey), and changes in *food preference*  
1035 *and reward* (Leeds Food Preference Questionnaire, LFPQ) [6] were also collected in this study via  
1036 the Qualtrics and E-Prime platforms respectively.

1037

1038 *Food cravings* were registered using a paper booklet based on the validated Control of Eating  
1039 Questionnaire [7].

1040

1041 The results of the LFPQ, Consumer Perspective Survey and cravings data will be published in a  
1042 separate paper.

1043

1044 **Blood sample collection procedures**

1045 Plasma samples for glucose analyses were collected in 3 ml VACUETTE fluoride-citrate tubes (ref.  
1046 454513), mixed by inversion 10 times and left at 4°C after which they were centrifuged within 1  
1047 h of collection at 1500 G for 10 min at 4°C. Serum samples for insulin, triglycerides, cholesterol  
1048 and liver function markers were collected in 5 ml VACUTAINER Gel serum tubes (ref. 367955),

1049 mixed by inversion and let to clot at room temperature for 30-60 min, after which they were  
1050 centrifuged at 1500 G for 10 min at 4°C.

1051

### 1052 **Interpretation of the percent energy compensation (%EC) value**

1053 An EC of 100% or close means effective compensation for the energy included in the preload. An  
1054 EC between 0 - 99% is referred to as “partial” or “incomplete compensation” that is, subjects  
1055 adjusted their intake later in the day by eating fewer calories after the control beverage, but this  
1056 reduction was below the difference between preload (breakfast + beverage) conditions. Values  
1057 <0 indicate ineffective compensation (i.e. the person did not adjust for any of the energy included  
1058 in the preload and consumed >105.6 kcal extra) [8].

1059

## 1060 **Additional Results**

### 1061 **Impact of sex and intervention site on glycaemic response**

1062 As expected (larger breakfast energy load in men), the 2-h curve and the 2-h iAUC for glucose  
1063 were higher in men than in women (effect of sex  $p<0.01$  in both glucose models).

1064

1065 Participants in Spain also showed higher glucose iAUCs than Danish participants, maybe due to  
1066 the slightly higher energy content of the breakfast (effect of intervention site  $p<0.01$ ). However,  
1067 intervention site did not impact the 2-h curve for glucose ( $p=0.081$ ).

1068

1069 Neither sex nor intervention site impacted on the insulin response.

### 1070 **Impact of sex, intervention site and age on beverage liking**

1071 Males and British plus Spanish participants rated beverages higher for *liking* than females and  
1072 Danish participants, who liked the beverages less (effect of sex  $p<0.001$ ; effect of intervention  
1073 site  $p<0.01$ ).

1074 Males and younger participants (18-45 y), rated beverages higher for *desire* than females and  
1075 older participants (46-60 y). Also, British participants rated all beverages higher for *desire* than  
1076 Danish and Spanish participants (effect of sex and age, both  $p<0.01$ ; effect of intervention site  
1077  $p<0.05$ ).

1078

1079

1080 **Impact of pleasantness (“Liking”) and wanting more beverage (“Desire for”) on appetite ratings**

- 1081 • There was no modulating action of *Liking* or *Desire for* on the impact of blend on hunger  
1082 ratings, which remained non-significant ( $p=0.119$  including *Liking*; and  $p=0.192$  including  
1083 *Desire for*).
- 1084 • There was no modulating action of *Liking* or *Desire* on the impact of blend on prospective  
1085 intake ratings, which remained significant ( $p<0.001$  including *Liking*; and  $p<0.001$   
1086 including *Desire for*).
- 1087 • In the fullness model, the *Liking* and *Desire for* variables both attenuated the impact of  
1088 blend on fullness, which went from significant ( $p=0.047$ ) to non-significant ( $p=0.056$  after  
1089 adding *Liking*; and  $p=0.052$  after adding *Desire for*).
- 1090 • In the desire to eat model, adding *Liking* did not change the results (impact of blend  
1091 remained significant,  $p=0.022$ ), however adding *Desire for* attenuated the impact of blend  
1092 on desire to eat, which went from significant ( $p=0.045$ ) to non-significant ( $p=0.052$ ).

1093

1094 **SUPPLEMENTARY TABLES**

1095 **Table S1.** Ingredient and nutritional information of the breakfast meals. Participants could choose  
 1096 between 2 items for fruit, spread, sweet food, and 3 items for the hot drink (choices were kept constant  
 1097 across CIDs). Decaffeinated versions for hot beverages were available.

Breakfast meal MEN	Spain		Denmark		UK	
	kJ	grams	kJ	grams	kJ	grams
Toasted, white bread	969	60	719	67	728	67
Low-fat cheese (cheddar-style)	495	45	330	45	326	46
Semi-skimmed milk (2% fat)	452	240	501	248	502	240
Fruit (apple or peaches), cored	409	180	389	180	392	177
Spread (butter or olive oil)	399	12	455	15	453	15
Sweet food (sugar or jam)	83	7	100	12	99	7
Hot drink prepared in water (Instant coffee/tea/herbal tea)	13	100	13	100	13	100
Total kJ (kcal) and grams	2820 (675)	644	2507 (600)	667	2513 (601)	652
Energy density (kJ/g)	4.4		3.8		3.9	
<b>Default breakfast (with apple, butter, coffee and sugar): 2504 kJ (599 kcal), 26 g protein (17.3 %E), 23 g fat (34.2 %E) and 77 g carbohydrates (51.1 %E), of which 6 g were fibre and 41 g sugars.</b>						
Breakfast meal WOMEN						
Toasted, white bread	678	42	513	48	510	47
Low-fat cheese (cheddar-style)	440	40	294	40	290	41
Semi-skimmed milk (2% fat)	282	150	303	150	314	150
Fruit (apple or peaches), cored	223	100	216	100	218	98
Spread (butter or olive oil)	276	8	303	10	299	10
Sweet food (sugar or jam)	80	7	100	12	99	7
Hot drink prepared in water (Instant coffee/tea/herbal tea)	13	100	13	100	13	100
Total kJ (kcal) and grams	1992 (477)	447	1742 (419)	460	1743 (417)	453
Energy density (kJ/g)	4.5		3.8		3.8	
<b>Default breakfast (with apple, butter, coffee and sugar): 1740 kJ (416 kcal), 20 g protein (18.8 %E), 16 g fat (34.0 %E) and 51 g carbohydrates (49.3 %E), of which 4 g were fibre and 27 g sugars.</b>						

1098

1099 **Table S2.** Socio-demographic characteristics of the sample based on total number of participants.

	All (n=60)	SP (n=22)	DK (n=21)	UK (n=17) <sup>a</sup>
Local residency (Yes)	95%	100%	86%	100%
Ethnicity				
White European	75%	77%	86%	59%
White non-European	0%	0%	0%	0%
East-Asian	12%	0%	5%	35%

**Table S2 (cont.)**

	<b>All (n=60)</b>	<b>SP (n=22)</b>	<b>DK (n=21)</b>	<b>UK (n=17)<sup>a</sup></b>
Other Asian origins	0%	0%	0%	0%
Black origin	3%	5%	0%	6%
Mixed	8%	14%	10%	0%
Any other ethnic group	2%	5%	0%	0%
<b>Minority ethnic group (Yes)</b>	<b>8%</b>	<b>5%</b>	<b>5%</b>	<b>18%</b>
<b>Highest education level attained</b>				
None	0%	0%	0%	0%
Primary	5%	5%	10%	0%
Secondary	25%	14%	48%	18%
Higher vocational school	13%	18%	10%	12%
University	57%	64%	33%	71%
<b>Marital status</b>				
Married/in partnership	35%	32%	38%	35%
Single (never married)	58%	68%	43%	65%
Separated or divorced	7%	0%	19%	0%
Widowed	0%	0%	0%	0%
<b>Household composition</b>				
Lives with children/ with other adults	100%	100%	100%	100%
Lives alone	0%	0%	0%	0%
<b>Employment status</b>				
Employed full-time	28%	27%	29%	29%
Employed part-time	15%	14%	19%	12%
Unemployed	15%	23%	19%	0%
Permanent sick leave	2%	5%	0%	0%
Carer	0%	0%	0%	0%
On full-time education	40%	32%	33%	59%
On training	0%	0%	0%	0%

1100 <sup>a</sup> Includes one female who dropped out after CID3 due to illness (COVID-19 diagnosis).

1101 Abbreviations: DK, Denmark intervention centre (University of Copenhagen); SP, Spain intervention centre  
 1102 (University of Navarra); UK, United Kingdom intervention centre (University of Liverpool).

1103

1104

1105 **Table S3.** Regression coefficients for the 2-h iAUC glucose model and Tukey’s adjusted post-hoc analyses. B  
 1106 estimates are the unweighted regression coefficients for the impact of blend on glucose iAUC values. Pr() indicates  
 1107 the p-value.

1108

1109 **B coefficient and 95% CI for B (reference: Sucrose) \***

	B Estimate	Std. Error	Pr(> t )	95% CI for B	
				Lower bound for B	Upper bound for B
StM_Mog	-191.2	131.1793	0.148	-447.089	64.800
StA_Tha	-335.195	131.1973	<b>0.012</b>	-591.284	-79.334
Suc_Ace	-354.866	131.1744	<b>0.008</b>	-610.816	-98.946

1110 \*Variables with overall impact on the 2-h iAUC glucose model: drink (p=0.028); intervention site (p=0.001); sex  
 1111 (p=0.008).

1112 **Table S3 (cont.)**

1113

**Post hoc Tukey test for the 2-h iAUC glucose model**

contrast	estimate	SE	df	t.ratio	p.value
Sucrose - StM_Mog	191.200	131.179	119.300	1.458	0.466
Sucrose - StA_Tha	335.195	131.198	119.012	2.555	0.057
Sucrose - Suc_Ace	354.866	131.174	118.813	2.705	0.039
StM_Mog - StA_Tha	143.996	131.225	119.798	1.097	0.692
StM_Mog - Suc_Ace	163.666	131.183	119.381	1.248	0.598
StA_Tha - Suc_Ace	19.671	131.192	119.023	0.150	0.999

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1115

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1117 **Table S4.** Regression coefficients for the 2-h iAUC insulin model and Tukey’s adjusted post-hoc analyses. B  
 1118 estimates are the unweighted regression coefficients for the impact of blend on insulin iAUC values. Pr() indicates  
 1119 the p-value.

1120

1121 **B coefficient and 95% CI for B (reference: Sucrose) \***

	B Estimate	Std. Error	Pr(> t )	95% CI for B	
				Lower bound for B	Upper bound for B
StM_Mog	-1413.9	305.7	<b>9.61E-06</b>	-2007.5	-815.0
StA_Tha	-1338.8	306.3	<b>2.67E-05</b>	-1937.0	-742.2
Suc_Ace	-1365.3	308.6	<b>2.16E-05</b>	-1968.3	-764.5

1122 \*Variables with overall impact on the 2-h iAUC insulin model: drink (p=0.000).

1123

**Post hoc Tukey test for the 2-h iAUC insulin model**

contrast	estimate	SE	df	t.ratio	p.value
<b>Sucrose - StM_Mog</b>	1413.861	305.738	119.300	4.624	<b>5.63E-05</b>
<b>Sucrose - StA_Tha</b>	1338.845	306.294	119.012	4.371	<b>0.000</b>
<b>Sucrose - Suc_Ace</b>	1365.263	308.579	118.813	4.424	<b>0.000</b>
StM_Mog - StA_Tha	-75.017	306.081	119.798	-0.245	0.995
StM_Mog - Suc_Ace	-48.598	308.257	119.381	-0.158	0.999
StA_Tha - Suc_Ace	26.418	308.760	119.023	0.086	0.999

1124

1125



1126 **Table S5.** Summary of blend impact on the lipaemic response (2 h curve). Results from linear mixed  
 1127 effects models adjusted for age group, sex, intervention site and breakfast energy intake.

Marker	Overall impact of blend on 2h curve	Details*
Triglycerides	p=0.371	<p><b>No differential impact of any S&amp;SE blend vs. sucrose</b></p> <p>Effect sizes (95%CI) vs. sucrose: StM_Mog 0.06 (-0.03, 0.16)            StA_Tha 0.08 (-0.01, 0.18)            StM_Mog -0.04 (-0.13, 0.06)</p>
Total cholesterol	p<0.001	<p><b>No differential impact of any S&amp;SE blend vs. sucrose.</b>            Small increase in total cholesterol values for StA_Tha vs. StM_Mog (p&lt;0.001 in post-hoc test):</p> <ul style="list-style-type: none"> <li>• Mean (SE) StM_Mog: 167.28 (2.13) mg/dL</li> <li>• Mean (SE) StA_Tha: 171.27 (2.44) mg/dL</li> <li>• Mean (SE) Suc_Ace: 168.69 (2.02) mg/dL</li> <li>• Mean (SE) Sucrose: 169.15 (2.09) mg/dL</li> </ul> <p>No pairwise comparisons vs. sucrose were significant (p&gt;0.01)</p> <p>Effect sizes (95%CI) vs. sucrose: StM_Mog -0.06 (-0.16, 0.03)            StA_Tha 0.04 (-0.06, 0.13)            StM_Mog -0.01 (-0.11, 0.09)</p>
LDL-cholesterol	p<0.001	<p><b>Increase in LDL-cholesterol for StA_Tha vs. Sucrose</b> (p&lt;0.001 in post-hoc test) and small increases for StA_Tha vs. StM_Mog and vs. Suc-Ace (both p&lt;0.001 in post-hoc tests):</p> <ul style="list-style-type: none"> <li>• Mean (SE) StM_Mog: 101.25 (1.85) mg/dL</li> <li>• Mean (SE) StA_Tha: 105.48 (2.08) mg/dL</li> <li>• Mean (SE) Suc_Ace: 102.40 (1.73) mg/dL</li> <li>• Mean (SE) sucrose: 102.48 (1.77) mg/dL</li> </ul> <p><b>% change after StA_Tha vs. sucrose 2.93% (p=0.000)</b></p> <p>No other pairwise comparisons vs. sucrose were significant (p&gt;0.01).</p> <p>Effect sizes (95%CI) vs. sucrose: StM_Mog -0.05 (-0.14, 0.05)            StA_Tha 0.11 (0.01, 0.20)            StM_Mog 0.00 (-0.10, 0.09)</p>
HDL-cholesterol	p<0.01	<p><b>Reduction in HDL-cholesterol for Suc_Ace vs. Sucrose</b> (p&lt;0.01 in post-hoc test):</p> <ul style="list-style-type: none"> <li>• Mean (SE) StM_Mog: 52.11 (0.91) mg/dL</li> <li>• Mean (SE) StA_Tha: 52.10 (0.84) mg/dL</li> <li>• Mean (SE) Suc_Ace: 51.87 (0.77) mg/dL</li> <li>• Mean (SE) sucrose: 53.1 (0.84) mg/dL</li> </ul> <p><b>% change after Suc_Ace vs. sucrose 2.32% (p=0.006)</b></p> <p>No other pairwise comparisons vs. sucrose were significant (p&gt;0.01)</p> <p>Effect sizes (95%CI) vs. sucrose: StM_Mog -0.08 (-0.17, 0.02)            StA_Tha -0.08 (-0.18, 0.01)            StM_Mog -0.10 (-0.20, -0.01)</p>

1128 SE, standard error.

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1130 \* Below are what are considered to be clinically significant changes for blood lipids, glucose, and insulin based on  
1131 the literature in **chronic studies** (Refs.[9–12]):

- 1132
- 1133 ➤ A change of 10% or more in total cholesterol, HDL-cholesterol and LDL-cholesterol (2h post-prandial mean
  - 1134 values)
  - 1135 ➤ A change of 30% minimum in triglycerides (2h post-prandial mean values)
  - 1136 ➤ A change of 30% or more in the 2h iAUC for glucose
  - 1137 ➤ A change of 30% or more in the 2h iAUC for insulin

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## 1140 CITATIONS

- 1141
- 1142 1. Garner, D.M.; Garfinkel, P.E. The Eating Attitudes Test: an index of the symptoms of anorexia nervosa.  
1143 *Psychol. Med.* **1979**, *9*, 273–9.
  - 1144 2. Masic, U.; Harrold, J.A.; Christiansen, P.; Cuthbertson, D.J.; Hardman, C.A.; Robinson, E.; Halford, J.C.G.  
1145 EffectS of non-nutritive sweetened beverages on appetITe during aCTive weiGht loss (SWITCH): Protocol  
1146 for a randomized, controlled trial assessing the effects of non-nutritive sweetened beverages compared to  
1147 water during a 12-week weight loss period and a. *Contemp. Clin. Trials* **2017**, *53*, 80–88,  
1148 doi:10.1016/j.cct.2016.12.012.
  - 1149 3. Booth, M. Assessment of physical activity: An international perspective. *Res. Q. Exerc. Sport* **2000**, *71*, 114–  
1150 120, doi:10.1080/02701367.2000.11082794.
  - 1151 4. PREVIEW Study The PREVIEW study- PREvention of diabetes through lifestyle Intervention and population  
1152 studies in Europe and around the World Available online: <http://preview.ning.com/> (accessed on Mar 21,  
1153 2017).
  - 1154 5. Vargas-Alvarez, M.A.; Al-Sehaim, H.; Brunstrom, J.M.; Castelnuovo, G.; Navas-Carretero, S.; Martínez, J.A.;  
1155 Almiron-Roig, E. Development and validation of a new methodological platform to measure behavioral,  
1156 cognitive, and physiological responses to food interventions in real time. *Behav. Res. Methods* **2022**, *1*–25,  
1157 doi:10.3758/s13428-021-01745-9.
  - 1158 6. Finlayson, G.; King, N.; Blundell, J. The role of implicit wanting in relation to explicit liking and wanting for  
1159 food: Implications for appetite control. *Appetite* **2008**, *50*, 120–127, doi:10.1016/j.appet.2007.06.007.
  - 1160 7. Dalton, M.; Finlayson, G.; Hill, A.; Blundell, J. Preliminary validation and principal components analysis of  
1161 the Control of Eating Questionnaire (CoEQ) for the experience of food craving. *Eur. J. Clin. Nutr.* **2015**, *69*,  
1162 1313–1317, doi:10.1038/ejcn.2015.57.
  - 1163 8. Almiron-Roig, E.; Palla, L.; Guest, K.; Ricchiuti, C.; Vint, N.; Jebb, S.A.; Drewnowski, A. Factors that  
1164 determine energy compensation: a systematic review of preload studies. *Nutr. Rev.* **2013**, *71*, 458–73,  
1165 doi:10.1111/nure.12048.
  - 1166 9. Tey, S.L.; Salleh, N.B.; Henry, J.; Forde, C.G. Effects of aspartame-, monk fruit-, stevia- and sucrose-  
1167 sweetened beverages on postprandial glucose, insulin and energy intake. *Int. J. Obes.* **2017**, *41*, 450–457,  
1168 doi:10.1038/ijo.2016.225.
  - 1169 10. Green, M.W.; Taylor, M.A.; Elliman, N.A.; Rhodes, O. Placebo expectancy effects in the relationship  
1170 between glucose and cognition. *Br. J. Nutr.* **2001**, *86*, 173–179, doi:10.1079/bjn2001398.
  - 1171 11. Bradley, R.; Kozura, E.; Buckle, H.; Kaltunas, J.; Tais, S.; Standish, L.J. Description of clinical risk factor  
1172 changes during naturopathic care for type 2 diabetes. *J. Altern. Complement. Med.* **2009**, *15*, 633–638,  
1173 doi:10.1089/acm.2008.0249.
  - 1174 12. American Diabetes Association Standards of medical care in diabetes - 2008. *Diabetes Care* **2008**, *31*, S12-  
1175 54.
- 1176  
1177  
1178