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

Eva Almiron-Roig[a-c], Santiago Navas-Carretero[a-d, (1)], Gabriele Castelnuovo[b (2)], Louise Kjølbæk[e], Ana Romo[a,b], Mie Normand[e], Niamh Maloney[f (2)], Charlotte A Hardman[f], Charo E Hodgkins[g], Hariklia Moshoyiannis[h], Graham Finlayson[i], Corey Scott[j], Monique M Raats[g], Joanne A Harrold[f], Anne Raben[e,k], Jason C G Halford[f,i], J Alfredo Martínez[a (2)]

[a]University of Navarra, Faculty of Pharmacy and Nutrition, Dept. of Food Science and Physiology, Pamplona, Spain
[b]University of Navarra, Center for Nutrition Research, Pamplona, Spain
[c]Navarra Institute for Health Research (IdiSNa), Pamplona, Spain
[d]Spanish Biomedical Research Centre in Physiopathology of Obesity and Nutrition (CIBERobn), Institute of Health Carlos III, Madrid, Spain
[e]Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, Copenhagen, Denmark
[f]Department of Psychology, University of Liverpool, Liverpool, UK
[g]Food Consumer Behaviour and Health Research Centre, School of Psychology, University of Surrey, Guildford, UK
[h]Bioiatriki Healthcare Group, Central Laboratory, Athens, Greece
[i]School of Psychology, University of Leeds, Leeds, UK
[j]Cargill R&D Centre Europe, Vilvoorde, Belgium
[k]Clinical Research, Copenhagen University Hospital – Steno Diabetes Center Copenhagen, Herlev, Denmark

[(1)] Author for correspondence: Santiago Navas-Carretero, Center for Nutrition Research, University of Navarra, 31008, Pamplona, Spain, snavas@unav.es) +34 948 425 600

[(2)] Present address for G Castelnuovo is University of Turin, Department of Medical Sciences, Turin, Italy; for N Maloney is Unilever Research and Development, Department of Consumer Technical Insight, Wirral, U.K., and for JA Martínez is IMDEA Food Institute, Madrid, Spain.

Author´s e-mail addresses in order of authorship: ealmiron@unav.es; snavas@unav.es; gabriele.castelnuovo@unito.it; louisekjoelbaek@nexs.ku.dk; aromo@unav.es; mino@nexs.ku.dk; niamh.maloney@liverpool.ac.uk; Charlotte.Hardman@liverpool.ac.uk; c.hodgkins@surrey.ac.uk; hmoshoyianni@bioiatriki.gr; G.S.Finlayson@leeds.ac.uk; Corey_Scott@cargill.com; m.raats@surrey.ac.uk; harrold@liverpool.ac.uk; ara@nexs.ku.dk; J.Halford@leeds.ac.uk; jalfmtz@unav.es.

Abstract

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

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

1. INTRODUCTION

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

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

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

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

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

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

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

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

2. METHODS

2.1. STUDY DESIGN

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

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

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

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

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

2.2. PARTICIPANTS

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

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

2.3. PROCEDURES

2.3.1. Screening session

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

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

2.3.2. Clinical Investigation Days

Figure 1 shows the procedures for the CIDs.

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

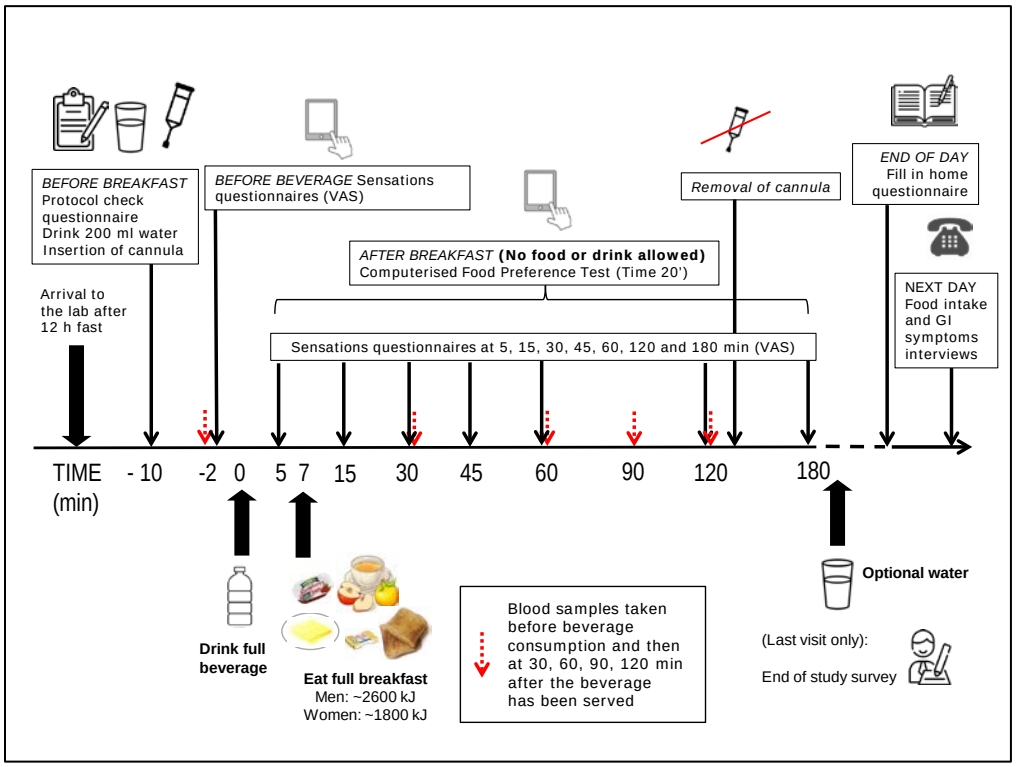


Figure 1. Clinical Investigation Day procedures. *Abbreviations*: GI, gastro-intestinal; VAS, visual analogue scale.

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

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

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

2.4. BREAKFAST AND TEST BEVERAGES

Table 1 lists the composition of each of the beverages used in the trials. Blends are hereafter referred to as: StM_Mog (stevia RebM 80% purity and Mogroside V 50% purity); StA_Tha (stevia

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

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

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

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

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

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

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
Thaumatin (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

2.5. DATA COLLECTION

2.5.1. Questionnaires

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

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

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

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

2.5.2. Gastro-intestinal symptoms interview

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

2.5.3. Dietary intake interview

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

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

2.6. BLOOD SAMPLING AND PROCESSING

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

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

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

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

2.7. DATA MANAGEMENT AND PROCESSING

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

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

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

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

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

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

2.8. SAMPLE SIZE AND STATISTICAL ANALYSES

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

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

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

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

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

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

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

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

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

3. RESULTS

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

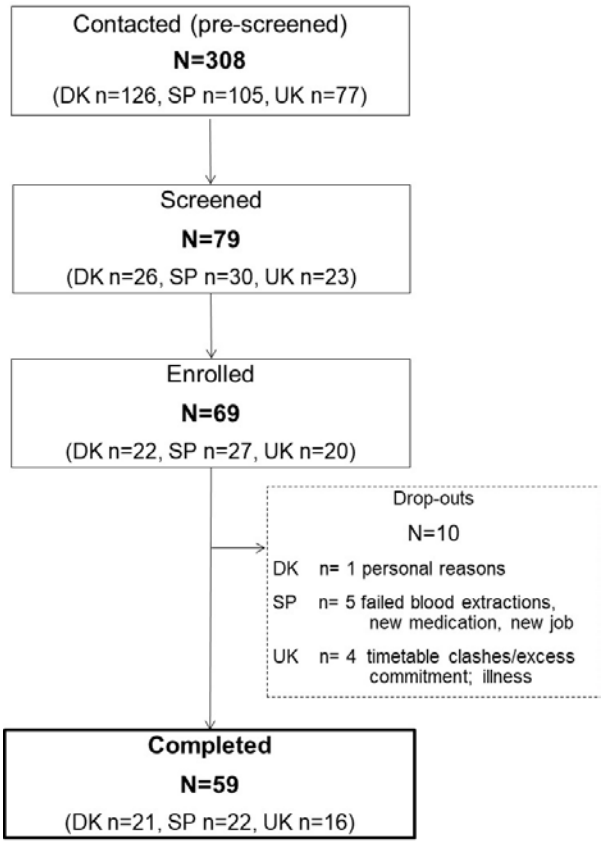


Figure 2. Recruitment flowchart for the Beverages multi-centre trial. Abbreviations: DK, Denmark (University of Copenhagen); SP, Spain (University of Navarra); UK, United Kingdom (University of Liverpool).

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

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

	All centres (N=60)[a]		**Spain (n=22)**		**Denmark (n=21)**		**U.K. (n=17)[a]**	
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^2)	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[b]	2.45	(1.3)	2.4	(1.4)	2.5	(1.3)	N/A	
Physical activity (IPAQ, Total MET-minutes/week)[c]	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)

[a] Includes one female who dropped out after CID3 due to illness (COVID-19 diagnosis).

[b] 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).

[c] Sample size for All centres N=45; Spain n=15; Denmark n=19; U.K. n=11.

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

3.1. Glycaemic impact

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

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

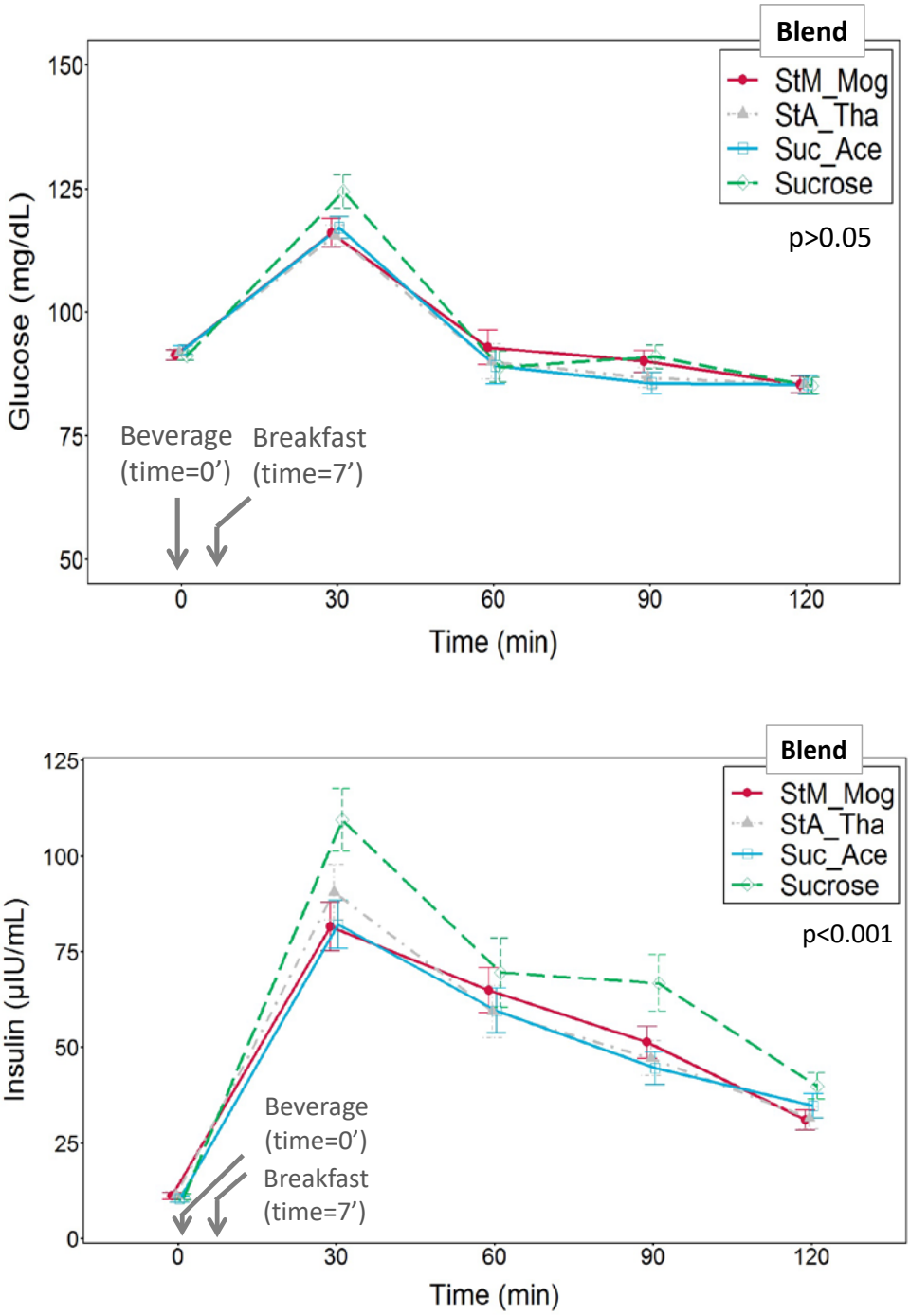


Figure 3. Fasting and post-prandial blood glucose (top) and insulin levels (bottom) across blend condition (N=42). Data points are means with SE. Overall impact of blend (linear mixed effects models results shown on the right upper corner.

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

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

[a] Linear mixed effects regression adjusted with intervention site, sex, age group and breakfast energy intake (intervention site and sex remained significant in the final glucose and TyG models).

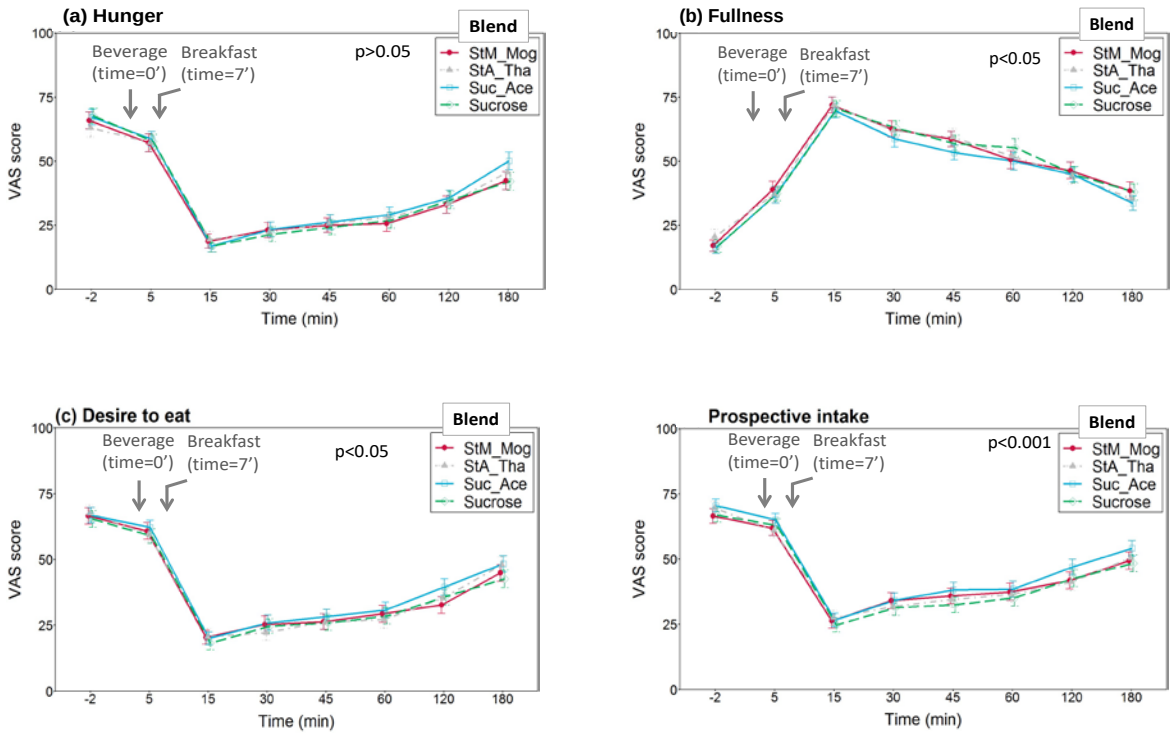
*An extreme value was detected for StA_Tha and for sucrose; plus, two for Suc_Ace. These values were excluded.

**An extreme value was detected for Suc_Ace and this value was excluded.

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

3.2. Appetite response

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



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.

3.3. Beverage liking and desire for more beverage scores

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

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

Table 4. Liking and desire scores for the intervention beverages collected at time 5 min (after drink consumption). Values are mean (SD) across all centres. Means with different superscript letters differ at 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[#]	59-60	59.53 (21.78)[a]	59.51 (23.95)[a]	71.32 (18.12)[b]	73.41 (16.90)[b]	p<0.001
Desire[$]	58-60	34.75 (23.12)[a]	32.97 (21.53)[a]	43.5 (25.62)[b]	47.07 (23.07)[c]	p<0.001

[*] One outlier was identified and excluded for sucrose for "Liking"; two missing values for "Desire" and one missing value for "Liking" (both for StA_Tha) were identified and those subjects were excluded.

[#] Linear mixed effects regression adjusted for intervention site, age group, and sex. Intervention site and sex retained a significant impact in the final model.

[$] Linear mixed effects regression adjusted for intervention site, age group and sexAll covariates retained a significant impact in the final model (see Supplementary Material, Additional Results, for details).

3.4. Energy and macronutrient intake

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

Mean (SD) total energy intakes by blend were: StM_Mog 11152 (86384) kJ; StA_Tha 11321 (86957) kJ; Suc_Ace 11283 (87399) kJ; sucrose 11480 (88922) kJ. These values were not statistically different in adjusted models, with the maximum difference corresponding to around 328 kJ (78 kcal) between the StM_Mog and the sucrose conditions.

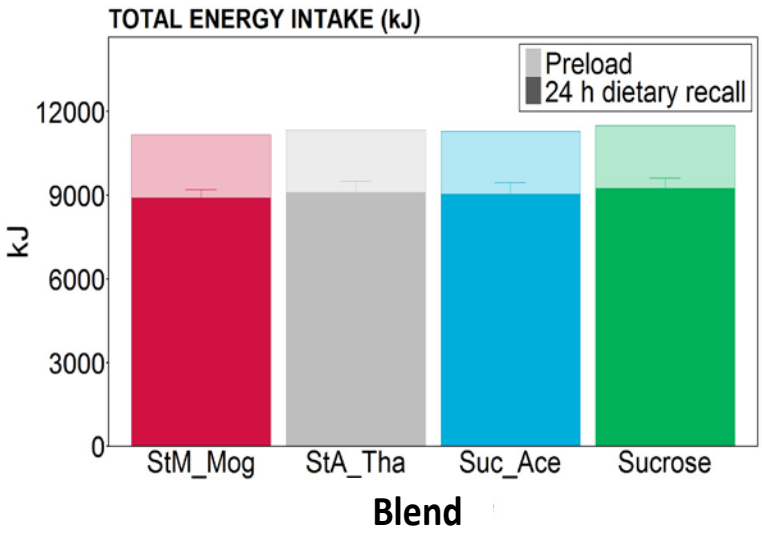


Figure 5. Total energy intake by blend condition. Data across all centres (N=59-60). Columns are total mean±SE energy consumed including preload (beverage plus breakfast) and 24 h *ad libitum* intake. There were no significant differences across blend in 24h *ad libitum* energy intake (p=0.278) , or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

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

3.4.1. Energy compensation

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

3.4.2. Twenty-four h ad libitum macronutrient intake

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

3.5. Safety parameters

3.5.1. Blood lipids

There was a small impact of some S&SE blends on some blood lipids, however, changes were of a very small magnitude (**Table S5**). Adjusted models showed an overall impact of blend on total and LDL-cholesterol (both $p<0.001$); and on HDL-cholesterol ($p<0.01$), but not on triglycerides ($p=0.371$). StA_Tha increased LDL-cholesterol levels by 2.9% vs sucrose ($p<0.001$), and increased total cholesterol vs StM_Mog ($p<0.001$) but not vs. sucrose ($p=0.076$). Also, compared with 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 sweetenerswere
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

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

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

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

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

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

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

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

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

4.1. Strengths and limitations

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

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

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

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

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

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

4.2. Conclusions

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

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

5. FUNDING, ACKNOWLEDGEMENTS AND CONFLICTS OF INTEREST

5.1. Acknowledgements

The authors are grateful to Tony Lam from NetUnion (Lausanne, Switzerland) for developing and managing the SWEET questionnaire delivery platform. To María Zabala, Salomé Pérez, M. Ángeles Vargas (University of Navarra), Sofie Frost (University of Copenhagen), and Eleni Triantafyllou (Bioiatriki), for technical assistance; and to Kristine Beaulieu and Catherine Gibbons (University of Leeds) for help with the randomization sequence and revisions to the protocol and manuscript. Data and images included in the Australian Health Survey food model booklet and related materials were used with permission from the Australian Bureau of Statistics (www.abs.gov.au).

5.2. Author´s contributions

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

5.3. Funding

SWEET has received funding from the EU's Horizon 2020 research & innovation programme (grant agreement 774293).

5.4. Data and code availability

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

5.5. Declaration of competing interest

JCGH, JAH, and CAH and are in receipt of research funding from the American Beverage Association; MMR and CEH's research centre provides consultancy to, and has received travel funds to present research results from organisations supported by food and beverage companies. ARA has received honoraria from Nestlé, Unilever and the International Sweeteners Association. CAH has received honoraria from the International Sweeteners Association. CS is an employee of Cargill, Inc. The other authors have nothing to declare.

REFERENCES

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

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

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

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

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

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-
764 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

Centers for Disease Control and Prevention. (n.d.). *National Health and Nutrition Examination Survey*. Retrieved March 30, 2017, from https://www.cdc.gov/nchs/nhanes/

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. L. Erlbaum Associates. https://books.google.es/books?id=2v9zDAsLvA0C&pg=PP1&redir_esc=y#v=onepage&q&f=false

Dalenberg, J. R., Patel, B. P., Denis, R., Veldhuizen, M. G., Nakamura, Y., Vinke, P. C., Luquet, S., & Small, D. M. (2020). Short-Term Consumption of Sucralose with, but Not without, Carbohydrate Impairs Neural and Metabolic Sensitivity to Sugar in Humans. *Cell Metabolism*, *31*(3), 493-502.e7. https://doi.org/10.1016/j.cmet.2020.01.014

Dotson, C. D., Zhang, L., Xu, H., Shin, Y. K., Vigues, S., Ott, S. H., Elson, A. E. T., Choi, H. J., Shaw, H., Egan, J. M., Mitchell, B. D., Li, X., Steinle, N. I., & Munger, S. D. (2008). Bitter taste receptors influence glucose homeostasis. *PLoS ONE*, *3*(12). https://doi.org/10.1371/journal.pone.0003974

Douglas, S. M., & Leidy, H. J. (2019). Novel Methodological Considerations Regarding the Use of Visual Analog Scale (VAS) Appetite Questionnaires in Tightly Controlled Feeding Trials. *Current Developments in Nutrition*, *3*(6), nzz061. https://doi.org/10.1093/CDN/NZZ061

Ebbeling, C. B., Feldman, H. A., Chomitz, V. R., Antonelli, T. A., Gortmaker, S. L., Osganian, S. K., & Ludwig, D. S. (2012). A Randomized Trial of Sugar-Sweetened Beverages and Adolescent Body Weight. *New England Journal of Medicine*, *367*(15), 1407–1416. https://doi.org/10.1056/NEJMoa1203388

EFSA. (2011). EFSA | European Food Safety Authority. Scientific Opinion on the substantiation of health claims related to intense sweeteners and contribution to the maintenance or achievement of a normal body weight (ID 1136, 1444, 4299), reduction of post-prandial gly. *EFSA Journal*, *9*(6). https://doi.org/10.2903/j.efsa.2011.2229

Feder, D. (2012). *How Sweet it is*. Prepared Foods. https://www.preparedfoods.com/articles/111643-how-sweet-it-is-

Finlayson, G., King, N., & Blundell, J. (2008). The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control. *Appetite*, *50*(1), 120–127. https://doi.org/10.1016/j.appet.2007.06.007

Flint, A., Raben, A., Blundell, J. E., & Astrup, A. (2000). Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders*, *24*(1), 38–48. http://www.ncbi.nlm.nih.gov/pubmed/10702749

Forestfield Software Ltd. (2021). *Dietplan7*. On-Line Software. http://dietplan7.com/index.html

Foster, E., Hawkins, A., Barton, K. L., Stamp, E., Matthews, J. N. S., & Adamson, A. J. (2017). Development of food photographs for use with children aged 18 months to 16 years: Comparison against weighed food diaries – The Young Person's Food Atlas (UK). *PLOS ONE*, *12*(2), e0169084. https://doi.org/10.1371/journal.pone.0169084

Gallagher, A. M., Ashwell, M., Halford, J. C. G., Hardman, C. A., Maloney, N. G., & Raben, A. (2021). Low-calorie sweeteners in the human diet: Scientific evidence, recommendations, challenges and future

needs. A symposium report from the FENS 2019 conference. In *Journal of Nutritional Science* (Vol. 10, p. e7). J Nutr Sci. https://doi.org/10.1017/jns.2020.59

Garner, D. M., & Garfinkel, P. E. (1979). The Eating Attitudes Test: an index of the symptoms of anorexia nervosa. *Psychological Medicine*, *9*(2), 273–279. http://www.ncbi.nlm.nih.gov/pubmed/472072

Gayoso-Diz, P., Otero-González, A., Rodriguez-Alvarez, M. X., Gude, F., García, F., De Francisco, A., & Quintela, A. G. (2013). Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: Effect of gender and age: EPIRCE cross-sectional study. *BMC Endocrine Disorders*, *13*, 47. https://doi.org/10.1186/1472-6823-13-47

Graaf, C. De, & Frijters, J. E. R. (1986). A psychophysical investigation of Beidler's mixture equation. *Chemical Senses*, *11*(3), 295–314. https://doi.org/10.1093/chemse/11.3.295

Green, M. W., Taylor, M. A., Elliman, N. A., & Rhodes, O. (2001). Placebo expectancy effects in the relationship between glucose and cognition. *British Journal of Nutrition*, *86*(2), 173–179. https://doi.org/10.1079/bjn2001398

Greylling, A., Appleton, K. M., Raben, A., & Mela, D. J. (2020). Acute glycemic and insulinemic effects of low-energy sweeteners: a systematic review and meta-analysis of randomized controlled trials. *Am J Clin Nutr*, *112*(4), 1002–1014. https://doi.org/doi: 10.1093/ajcn/nqaa167

Grotz, V. L., & Jokinen, J. D. (2014). Sucralose affects glycemic and hormonal responses to an oral glucose load. In *Diabetes Care* (Vol. 37, Issue 6, pp. e148–e148). American Diabetes Association. https://doi.org/10.2337/dc13-2972

Healthcare Software Solutions S.A. (2021). *Nutrium | All-in-one Nutrition Software: Nutrition Analysis & CRM*. On-Line Software. https://nutrium.com/en

Higgins, K. A., & Mattes, R. D. (2019). A randomized controlled trial contrasting the effects of 4 low-calorie sweeteners and sucrose on body weight in adults with overweight or obesity. *American Journal of Clinical Nutrition*, *109*(5), 1288–1301. https://doi.org/10.1093/ajcn/nqy381

Hill, A. J., & Blundell, J. E. (1982). Nutrients and behaviour: Research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. *Journal of Psychiatric Research*, *17*(2), 203–212. https://doi.org/10.1016/0022-3956(82)90023-1

Jiménez-Domínguez, G., Ble-Castillo, J. L., Aparicio-Trápala, M. A., Juárez-Rojop, I. E., Tovilla-Zárate, C. A., Ble-Castillo, D. J., García-Vázquez, C., Olvera-Hernández, V., Pérez-Pimienta, B., Diaz-Zagoya, J. C., & Mendez, J. D. (2015). Effects of acute ingestion of native banana starch on glycemic response evaluated by continuous glucose monitoring in obese and lean subjects. *International Journal of Environmental Research and Public Health*, *12*(7), 7491–7505. https://doi.org/10.3390/ijerph120707491

Jones, B., & Kenward, M. G. (2015). *Design and Analysis of Cross-Over Trials* (3rd ed.). Chapman and Hall/ CRC. https://www.amazon.com/Analysis-Cross-Over-Monographs-Statistics-Probability/dp/1439861420

Kassambara, A. (2022, November 9). *Pipe-Friendly Framework for Basic Statistical Tests [R package rstatix version 0.7.1]*. Comprehensive R Archive Network (CRAN). https://cran.r-

project.org/package=rstatix

Khan, T. A., & Sievenpiper, J. L. (2021). Low-Calorie Sweeteners with Carbohydrate Do Not Impair Insulin Sensitivity in Humans: Re-analysis Highlighting the Importance of the Comparator. *Cell Metabolism*, *33*(2), 225–226. https://doi.org/10.1016/J.CMET.2020.10.024

Kim, S. K., Chen, Y., Abrol, R., Goddard, W. A., & Guthrie, B. (2017). Activation mechanism of the G protein-coupled sweet receptor heterodimer with sweeteners and allosteric agonists. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(10), 2568–2573. https://doi.org/10.1073/pnas.1700001114

Kraftaerk Foodtech. (n.d.). *Dankost Pro er anerkendt af hele fødevarebranchen - læs mere her*. On-Line Software. Retrieved September 22, 2021, from https://www.kraftvaerk-foodtech.com/da

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for t-tests and ANOVAs. *Frontiers in Psychology*, *4*(NOV), 863. https://doi.org/10.3389/fpsyg.2013.00863

Lee, H. Y., Jack, M., Poon, T., Noori, D., Venditti, C., Hamamji, S., & Musa-Veloso, K. (2021). Effects of Unsweetened Preloads and Preloads Sweetened with Caloric or Low-/No-Calorie Sweeteners on Subsequent Energy Intakes: A Systematic Review and Meta-Analysis of Controlled Human Intervention Studies. In *Advances in Nutrition* (Vol. 12, Issue 4, pp. 1481–1499). Adv Nutr. https://doi.org/10.1093/advances/nmaa157

Malik, V. S., & Hu, F. B. (2022). The role of sugar-sweetened beverages in the global epidemics of obesity and chronic diseases. In *Nature Reviews Endocrinology* (Vol. 18, Issue 4, pp. 205–218). Nat Rev Endocrinol. https://doi.org/10.1038/s41574-021-00627-6

Michail, N. (2017). *Monk fruit-stevia blends are still best for natural sugar reduction, says Layn*. Food Navigator,. https://www.foodnavigator.com/Article/2017/12/05/Monk-fruit-stevia-blends-are-still-best-for-natural-sugar-reduction-says-Layn#

Mora, M. R., & Dando, R. (2021). The sensory properties and metabolic impact of natural and synthetic sweeteners. In *Comprehensive Reviews in Food Science and Food Safety* (Vol. 20, Issue 2, pp. 1554–1583). John Wiley & Sons, Ltd. https://doi.org/10.1111/1541-4337.12703

Movahedian, M., Golzan, S. A., Ashtary-Larky, D., Clark, C. C. T., Asbaghi, O., & Hekmatdoost, A. (2021). The effects of artificial- and stevia-based sweeteners on lipid profile in adults: a GRADE-assessed systematic review, meta-analysis, and meta-regression of randomized clinical trials. In *Critical Reviews in Food Science and Nutrition* (pp. 1–17). Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2021.2012641

Nichol, A. D., Holle, M. J., & An, R. (2018). Glycemic impact of non-nutritive sweeteners: A systematic review and meta-Analysis of randomized controlled trials. In *European Journal of Clinical Nutrition* (Vol. 72, Issue 6, pp. 796–804). Eur J Clin Nutr. https://doi.org/10.1038/s41430-018-0170-6

Nordic Council of Ministers. (n.d.). *Nordic nutrition recommendations 2012 : integrating nutrition and physical activity*.

O'Connor, D., Pang, M., Castelnuovo, G., Finlayson, G., Blaak, E., Gibbons, C., Navas-Carretero, S.,

Almiron-Roig, E., Harrold, J., Raben, A., & Martinez, J. A. (2021). A rational review on the effects of sweeteners and sweetness enhancers on appetite, food reward and metabolic/adiposity outcomes in adults. In *Food and Function* (Vol. 12, Issue 2, pp. 442–465). https://doi.org/10.1039/d0fo02424d

Pawar, R. S., Krynitsky, A. J., & Rader, J. I. (2013). Sweeteners from plants-with emphasis on Stevia rebaudiana (Bertoni) and Siraitia grosvenorii (Swingle). In *Analytical and Bioanalytical Chemistry* (Vol. 405, Issue 13, pp. 4397–4407). Springer Verlag. https://doi.org/10.1007/s00216-012-6693-0

Pepino, M. Y., Tiemann, C. D., Patterson, B. W., Wice, B. M., & Klein, S. (2013). Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care*, *36*(9), 2530–2535. https://doi.org/10.2337/dc12-2221

Public Health England. (2020). *NDNS: results from years 9 to 11 (2016 to 2017 and 2018 to 2019)*. National Diet and Nutrition Survey. https://www.gov.uk/government/statistics/ndns-results-from-years-9-to-11-2016-to-2017-and-2018-to-2019

Rios-Leyvraz, M., & Montez, J. (2022). *Health effects of the use of non-sugar sweeteners: a systematic review and meta-analysis. Geneva: World Health Organization*. World Health Organization,. https://www.who.int/publications/i/item/9789240046429

Robinson, E., Nolan, S., Tudur-Smith, C., Boyland, E. J., Harrold, J. A., Hardman, C. A., & Halford, J. C. (2014). Will smaller plates lead to smaller waists? A systematic review and meta-analysis of the effect that experimental manipulation of dishware size has on energy consumption. *Obes Rev*, *15*(10), 812–821. https://doi.org/10.1111/obr.12200

Rogers, P J, Hogenkamp, P. S., de Graaf, C., Higgs, S., Lluch, A., Ness, A. R., Penfold, C., Perry, R., Putz, P., Yeomans, M. R., & Mela, D. J. (2016). Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *International Journal of Obesity (2005)*, *40*(3), 381–394. https://doi.org/10.1038/ijo.2015.177

Rogers, Peter J., & Hardman, C. A. (2015). Food reward. What it is and how to measure it. *Appetite*, *90*, 1–15. https://doi.org/10.1016/j.appet.2015.02.032

Ruiz, E., Rodriguez, P., Valero, T., Ávila, J. M., Aranceta-Bartrina, J., Ángel, G., González-Gross, M., Ortega, R. M., Serra-majem, L., & Varela-moreiras, G. (2017). Dietary Intake of Individual (Free and Intrinsic) Sugars and Food Sources in the Spanish Population : Findings from the ANIBES Study. *Nutrients*, *9*(275). https://doi.org/10.3390/nu9030275

Schiffman, S. S., Sattely-Miller, E. A., Graham, B. G., Zervakis, J., Butchko, H. H., & Stargel, W. W. (2003). Effect of repeated presentation on sweetness intensity of binary and ternary mixtures of sweeteners. *Chemical Senses*, *28*(3), 219–229. https://doi.org/10.1093/chemse/28.3.219

Simental-Mendía, L. E., Rodríguez-Morán, M., & Guerrero-Romero, F. (2008). The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. *Metabolic Syndrome and Related Disorders*, *6*(4), 299–304. https://doi.org/10.1089/met.2008.0034

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

980

981

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.

Pre-study sensory analysis

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

Additional questionnaires information

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

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

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

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

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

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

Blood sample collection procedures

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

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

Interpretation of the percent energy compensation (%EC) value

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

Additional Results

Impact of sex and intervention site on glycaemic response

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

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

Neither sex nor intervention site impacted on the insulin response.

Impact of sex, intervention site and age on beverage liking

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

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

Impact of pleasantness ("Liking") and wanting more beverage ("Desire for") on appetite ratings

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

SUPPLEMENTARY TABLES

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

	Spain		Denmark		UK	
Breakfast meal MEN	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	
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.						
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	
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.						

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)[a]
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.)

	All (n=60)	SP (n=22)	DK (n=21)	UK (n=17)[a]
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%
Minority ethnic group (Yes)	8%	5%	5%	18%
Highest education level attained				
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%
Marital status				
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%
Household composition				
Lives with children/ with other adults	100%	100%	100%	100%
Lives alone	0%	0%	0%	0%
Employment status				
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%

[a] Includes one female who dropped out after CID3 due to illness (COVID-19 diagnosis).

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

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

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

				95% CI for B	
	B Estimate	Std. Error	Pr(>|t|)	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	**0.012**	-591.284	-79.334
Suc_Ace	-354.866	131.1744	**0.008**	-610.816	-98.946

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

Table S3 (cont.)

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

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

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

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

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

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

contrast	estimate	SE	df	t.ratio	p.value
Sucrose - StM_Mog	1413.861	305.738	119.300	4.624	**5.63E-05**
Sucrose - StA_Tha	1338.845	306.294	119.012	4.371	**0.000**
Sucrose - Suc_Ace	1365.263	308.579	118.813	4.424	**0.000**
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

Table S5. Summary of blend impact on the lipaemic response (2 h curve). Results from linear mixed 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	**No differential impact of any S&SE blend vs. sucrose** 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)
Total cholesterol	p<0.001	**No differential impact of any S&SE blend vs. sucrose.** Small increase in total cholesterol values for StA_Tha vs. StM_Mog (p<0.001 in post-hoc test): • Mean (SE) StM_Mog: 167.28 (2.13) mg/dL • Mean (SE) StA_Tha: 171.27 (2.44) mg/dL • Mean (SE) Suc_Ace: 168.69 (2.02) mg/dL • Mean (SE) Sucrose: 169.15 (2.09) mg/dL No pairwise comparisons vs. sucrose were significant (p>0.01) 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)
LDL-cholesterol	p<0.001	**Increase in LDL-chol for StA_Tha_ vs. Sucrose** (p<0.001 in post-hoc test) and small increases for StA_Tha vs. StM_Mog and vs. Suc-Ace (both p<0.001 in post-hoc tests): • Mean (SE) StM_Mog: 101.25 (1.85) mg/dL • Mean (SE) StA_Tha: 105.48 (2.08) mg/dL • Mean (SE) Suc_Ace: 102.40 (1.73) mg/dL • Mean (SE) sucrose: 102.48 (1.77) mg/dL **% change after StA_Tha vs. sucrose 2.93% (p=0.000)** No other pairwise comparisons vs. sucrose were significant (p>0.01). 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)
HDL-cholesterol	p<0.01	**Reduction in HDL-chol for Suc_Ace vs. Sucrose** (p<0.01 in post-hoc test): • Mean (SE) StM_Mog: 52.11 (0.91) mg/dL • Mean (SE) StA_Tha: 52.10 (0.84) mg/dL • Mean (SE) Suc_Ace: 51.87 (0.77) mg/dL • Mean (SE) sucrose: 53.1 (0.84) mg/dL **% change after Suc_Ace vs. sucrose 2.32% (p=0.006)** No other pairwise comparisons vs. sucrose were significant (p>0.01) 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)

SE, standard error.

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

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

CITATIONS

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