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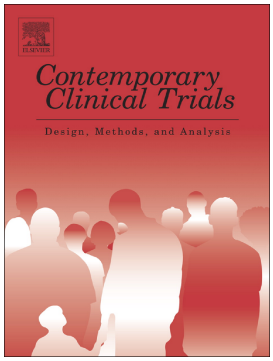

EffectS of non-nutritive sWeetened beverages on appetITe during aCtive weigHt loss (SWITCH): Protocol for a randomized, controlled trial assessing the effects of non-nutritive sweetened beverages compared to water during a 12-week weight loss period and a follow up weight maintenance period

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Effect*S* of non-nutritive s*W*eetened beverages on appet*I*te during a*C*tive weig*H*t 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 follow up weight maintenance period.

Masic, U., Harrold, J.A., Christiansen, P., Cuthbertson, D., Hardman, C., Robinson, E., & Halford, J.C.G.

Abstract

Background: Acute and medium-term intervention studies suggest that non-nutritive sweeteners (NNS) are beneficial for weight loss, however there is limited human data on the long-term effects of consuming NNS on weight loss, maintenance, and appetite. Further research is therefore required to elucidate the prolonged impact of NNS consumption on these outcome measures.

Methods/Design: A randomized parallel groups design will be used to assess whether regular NNS beverage intake is equivalent to a water control in promoting weight loss over 12-weeks (weekly weight loss sessions; Phase I), then supporting weight maintenance over 40-weeks (monthly sessions; Phase II) and subsequently independent weight maintenance over 52-weeks (Phase III) in 432 participants. A subset of these participants (n=116) will complete laboratory-based appetite probe days (15 sessions; 3 sessions each at baseline, at the start of phase I and the end of each phase). A separate subset (n= 50) will complete body composition scans (DXA) at baseline and at the end of each phase. All participants will regularly be weighed and will complete questionnaires and cognitive tasks to assess changes in body weight and appetitive behaviours. Measures of physical activity and biochemical markers will also be taken.

Discussion: The trial will assess the efficacy of NNS beverages compared to water during a behavioural weight loss and maintenance programme. We aim to understand whether the impact of NNS on weight, dietary adherence and well-being are beneficial or transient and effects on prolonged successful weight loss and weight maintenance through sustained changes in appetite and eating behaviour.

Trial Registration: Clinical Trials: NCT02591134; registered: 23.10.2015

Keywords: Weight loss; obesity; low calorie sweeteners (LCS); non-nutritive sweeteners (NNS); body weight; sugar-sweetened beverages (SSB)

Introduction

The global rise in the prevalence of obesity is a major public health concern [1] attributed to an 'obesogenic environment', characterised by increases in low-cost, energy-dense foods and drinks, amongst other lifestyle factors. Increased consumption of sugar-sweetened beverages (SSBs) has been related to this rise in obesity prevalence [2, 3, 4], possibly due to the poor satiating value of these beverages [5, 6], with individuals not responding to additional calories physiologically [7] or cognitively [8, 9]

Non-nutritive sweeteners (NNS) enable the consumption of sweet tasting, yet low energy, foods and beverages. Low energy foods and beverages could help reduce energy intake due to an absence of energy compensation [11, 12] driven by changes in appetite regulation and gut hormone release compared to SSB [12]. Consumption of these products has been associated with reductions in body weight and weight-related disease risk [13]. Recently, Peters et al., [14, 15] provided participants with either NNS beverages or water as part of a 12-week behavioural weight loss programme to understand the effects of NNS during active weight loss [14], followed by a weight maintenance period [15]. In the longest study to date, the NNS group lost significantly more weight and reported greater reductions in hunger after 12 weeks. This group was also better able to maintain weight loss over the subsequent 40 weeks compared to control. These data indicate potential benefits of including NNS products to help reduce and maintain reduced body weight, although no mechanisms for these effects were examined. Indeed, recent reviews of randomized controlled clinical trials (RCTs) show associations between substituting SSBs for NNS and lower weight, fat mass, waist circumference and body mass index (BMI) in both adults and children [16]; long-term lower energy intake and weight loss (when combined with other low energy alternatives) [17] and no relation between NNS and increased energy intake or weight [18]

Despite promising findings, concerns remain about the potential negative impact of NNS beverages on appetite and weight management. Some argue that chronic NNS exposure may undermine energy regulation by impeding the learned association between sweetness and energy [19, 20]. Rodent studies suggest that NNS alter blood glucose levels via altered post-prandial glucagon-like peptide 1 (GLP-1) secretion, weakening the impact of sugars on the satiety hormone response [21], while clinical studies of insulin sensitivity and GLP-1 release yield mixed results [22, 23, 24, 25]. Some epidemiological evidence suggest an association between the use of NNS and obesity [26, 13, 27], though findings may be explained by reverse causation [18, 27].

The mechanisms by which NNS may affect energy balance and weight maintenance during a long-term intervention still need to be elucidated, including the influence on energy intake, compensation, appetite control, and food choice which has not been quantified thus far. The present research will examine (i) the effect of NNS beverages on weight loss and long-term weight maintenance and (ii) the behavioural and biological mechanisms through which these effects arise as compared to water.

Methods and Materials

Outcome Measures

Primary Outcome Measure

The primary outcome measure will be weight change (kg) from baseline to post-weight loss phase (WLP) and weight maintenance phase (WMP; end of year one).

Secondary Outcome Measures

Physiological

i) Anthropometric (waist and hip circumference) and body composition (percentage fat mass and total fat mass, upper body and lower body fat and fat-free mass measurements using DXA scanning in Subset 2).
ii) Biochemical changes in markers of glycaemic control (fasting insulin, fasting glucose and HbA1c), fasting lipid profiles (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL)) and liver function tests (ALT, AST and GGT)).

Psychological

i) Measurements of appetite expression.

   Changes in food preferences, cravings and eating behaviours (through 24-hour recall food diaries, sweet food frequency questionnaires (sFFQ) and associated craving questionnaires) as well as changes in mood and mental well-being will also be examined in the study population as a whole. Appetite expression (hunger, satiety, liking and wanting) and calorie intake (food choice, macronutrient intake and energy compensation) will be assessed in a subset containing equal numbers of NNS beverage and water consumers (Subset 1). These between-group (and appropriate within-group) comparisons will determine whether the effects of NNS or water on subjective ratings of appetite and mood are beneficial to successful weight management (over the weight loss and weight maintenance phases). Examination of food choice, changes in attitudes to NNS or water, food preference, cravings and macronutrient intake will investigate potential detrimental or beneficial in preference for sweetness.

ii) Experience and ease of weight loss in WLP and assisted WMP.

   To determine the behavioural mechanisms of action when using NNS or water during weight loss and weight loss maintenance in those who regularly consume NNS and/or water beverages, the experience of and perceived ease of weight management will be assessed by the inclusion of psychological cognitive tests and questionnaires.

Trial Design

The study will use a parallel randomized design with two arms: an NNS beverage arm (active) or a water beverage arm (control). The trial will enrol 432 participants. All participants will undergo an initial 12-week weight loss phase (WLP; Phase I) - in which participants will attend weekly behavioural weight loss group sessions and incorporate either NNS or water as their treatment group beverages into their diet throughout the trial. Subsequently, participants will undergo a 40-week assisted weight maintenance phase (WMP; Phase II) and attend monthly group sessions (whilst continuing to consume NNS or water). A third phase will consist of a further 12-month non-assisted WMP (Phase III) in which participants will not receive any further behavioural and nutritional advice, but continue to consume NNS beverages or water and complete monthly retrospective appetite and mood ratings (**Figure 1**). Two subgroups will additionally complete appetite probe days (Subset 1; 15 sessions in total; n=116) or undergo DXA scanning (Subset 2; 4 sessions in total; n=50).

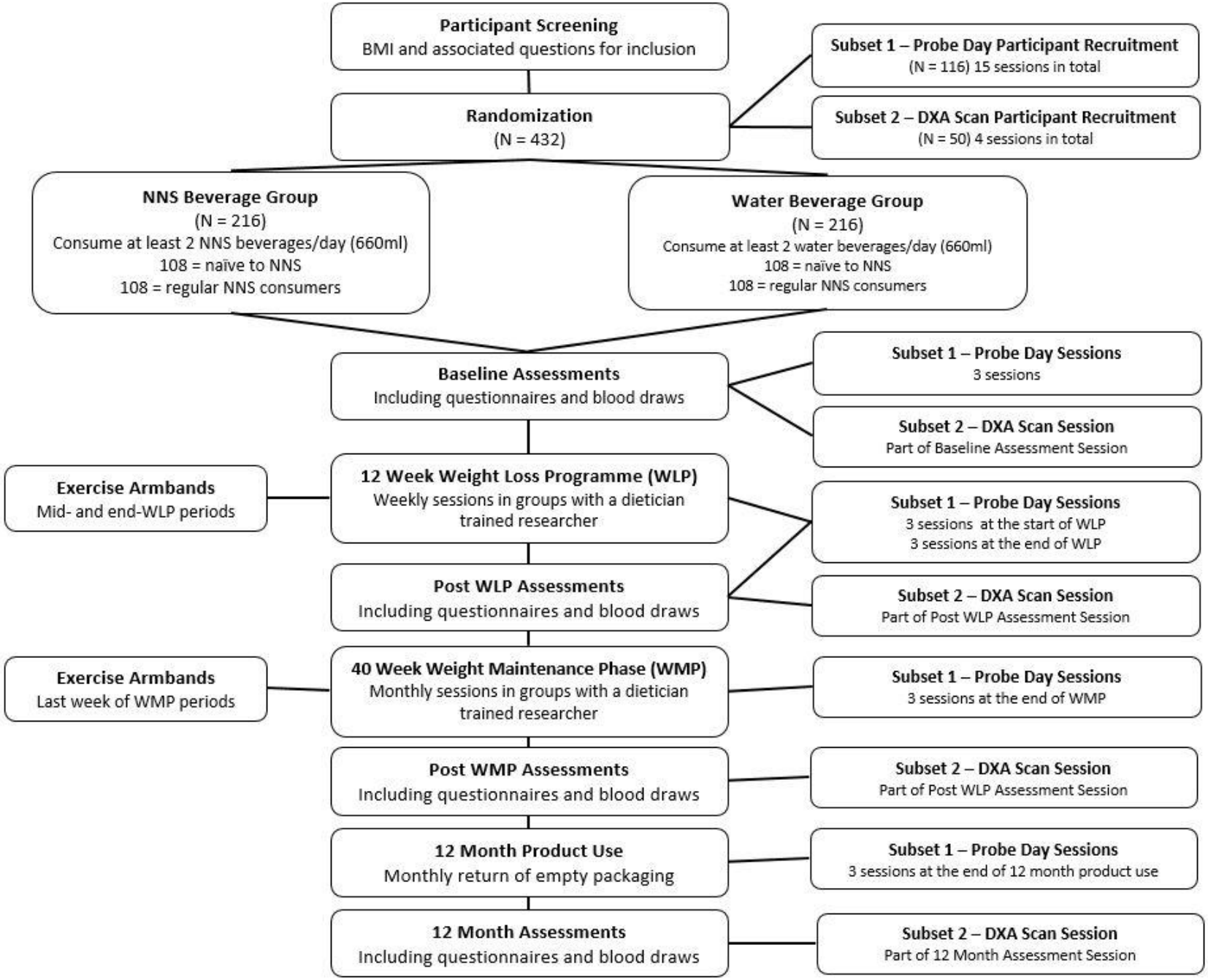

Figure 1. Study flow diagram. NNS=Non-nutritive sweetened

Participants

Inclusion Criteria

The following inclusion criteria must be met: healthy overweight and obese adult males and females (BMI: 27-35kg/m^2; age range: 18-65y) who are either regular consumers (>3 drinks per week but <2 litres per day) of chilled NNS or are NNS beverage naïve (assessed as no NNS or up to 25% NNS beverage choices made over the last five years). Participants must accept randomization to either a water or NNS beverage condition and must be willing to abstain from consuming NNS beverages (if randomized to the water condition) or willing to consume NNS beverages (if randomised to NNS group) for the duration of the trial. They must not be planning on moving from the Merseyside area for the duration of their participation in the trial and they must provide voluntary written informed consent.

Exclusion Criteria

Exclusion criteria include: expressing a significant dislike of NNS beverages (less than moderately likeable on a 7-point Likert scale); first year University students (to avoid the 'freshman 15' phenomenon of weight gain within the first year of attending University and thus increasing variance in the sample; [29, 30]); any significant health problems (including a history of cardiovascular diseases); any gastro-intestinal problems;; type 1 or type 2 diabetes mellitus; taking any medication or supplements known to influence appetite or weight within the past month and/or during the study; stated major psychiatric disorder (made clear by medicines specified); currently suffering from depression (made clear by medicines specified); currently being treated for a psychological condition; pregnancy, planning to become pregnant, breastfeeding or less than 6 months post-partum; history of anaphylaxis to food; known food allergies or food intolerance; dislike of >25% of the study foods offered on the appetite probe day (Subset 1 only); non-breakfast eaters; smoking or having ceased smoking in the last six months; currently dieting, having ceased a diet in less than 4 weeks, or having lost significant amounts of weight in the previous year; engaging in regular intense exercise (>2 hours per week); having significantly changed their physical activity patterns in the past 4 weeks or intending to change them during the study (other than to adhere to advice given in the behavioural intervention); inability to adhere to a defined exercise programme such as inability to increase physical activity; receiving systemic or local treatment likely to interfere with evaluation of the study parameters; working in appetite or feeding-related disciplines; being on specific food avoidance diets; having had bariatric surgery; having abnormal eating behaviour (restrained eaters measured by the Dutch Eating Behaviour Questionnaire Restraint Scale [DEBQ-R] with a cut-off point of more than 4 [31]);

and binge eaters measured by the Binge Eating Scale [BES] with a cut-off point of 27 or over [32]). Only one individual per household will be eligible for inclusion.

Recruitment

Participants will be recruited from the Merseyside area of England. Volunteers who respond to the study advertisements (verbal advertisements e.g. radio; text advertisements e.g. newspapers and posters; and social media e.g. Facebook and Twitter) will be given full information on the study and the process of informed consent will be undertaken. All measurements will be conducted by trained staff in the University of Liverpool. Recruitment will commence in July 2016.

Randomization

Participants will be randomized to condition (NNS or water) after their initial screening session. This will be stratified using block randomization (random block sizes of 4 and 6) to ensure equal numbers of participants are randomised to each arm (1:1) using a computer-generated randomisation sequence. Randomisation strata will be based on sex (male/female), age (19-34y; 35-50y; 51-65y), BMI (<30kg/m^2 and >30kg/m^2) and beverage consumption (NNS naïve or regular NNS consumption). Participants from the same household will not be accepted into the trial to account for likely data clustering. Randomization will be carried out by an academic investigator who is not involved in the implementation of the trial and will be conducted using a database only seen by this academic investigator. Once the participant has passed the screening stage, the academic investigator will be provided with the strata required to allocate condition and the researcher will inform the participant upon their second visit to the laboratory.

Randomisation to each group will also be completed for the subsets of participants taking part in the appetite probe day sessions (Subset 1) or DXA scans (Subset 2) with equal numbers of NNS and water control participants in each subset.

Withdrawal

Participation in the study is voluntary, and participants may withdraw from the study without providing a reason. If a participant chooses to withdraw, every effort will be made to complete early termination assessments if applicable. Any participant who has completed two weeks or more of the WLP and decides to discontinue will be encouraged to undergo an early termination visit if willing. The participant will be contacted on three occasions and if no response is made the participant will be excluded from further measures.

Intervention

Beverage condition

NNS participants will be asked to consume at least two servings (2 x 330ml) of NNS beverage per day (still or carbonated) during the trial. Control participants will be asked to consume at least two servings (2 x 330ml) of water per day (still or carbonated) and will be asked to refrain from consuming NNS beverages. To minimise participant burden, beverages will be supplied using a home shopping delivery-style service with participants selecting beverages from a pre-defined list to be delivered to their home.

Adherence

Compliance will be assessed using an online daily beverage log of all drinks consumed (including detail of volume and energy content) throughout the trial. Similarly, participants will be periodically asked to return empty packaging of their elected drinks during the WLP, assisted WMP and non-assisted WMP. 24-hour dietary recalls will be completed at various stages throughout the trial and can be utilized as an additional adherence measure.

Adverse Events

Overall, no immediate risk is anticipated with the current study. Indeed, a previous study has utilized a similar design with no negative outcomes to participants [14, 15]. Nevertheless, the monitoring of adverse events will be carried out at monthly intervals throughout the trial.

Use of NNS

The sweeteners in the NNS beverages provided have been approved by the European Food Safety Authority (EFSA) and are safe at the doses to be consumed. The tolerable daily intake (TDI) of aspartame, for example, is 40mg/kg body weight per day [33]. For an overweight female weighing 80kg (height 168cm; BMI 28.6kg/m^2) this would be 3200mg on a daily basis. To put this into perspective, the most one NNS beverage portion (330ml) is likely to provide is 192mg of aspartame. For this individual 16.6 cans (or 5,478 ml) NNS beverage would need to be consumed each day – a quantity far exceeding that provided to participants in this trial - to achieve the TDI.

Measurements and visits

Psychological measurements

A range of questionnaires and cognitive tests assessing secondary outcomes of appetite, craving and mood will be completed at baseline and at the end of each phase of the trial -

the WLP (Phase I), assisted WMP (Phase II) and at the end of the non-assisted WMP (Phase III). For appetite, craving and eating behaviours (including food choice) these include: the *Three Factor Eating Questionnaire* (TFEQ; [34]) measuring eating behaviours; *Power of Food Scale* (PoF; [35]) measuring differences in hedonic hunger; *Eating Self Efficacy* and *Weight Self Efficacy* [36] measuring self-perceived ability to control eating and weight; *Attitudes towards NNS Beverages and Water* measuring participant's beliefs and attitudes about these beverages; *Craving for Sweet Foods Questionnaire* assessing desire for sweet foods; the *7-day (and 24-hour for Subset 1) Control of Eating Questionnaire* assessing specific food cravings; *mini-IPIP* personality trait measure [37]; *Self-reported Eating Rate* [38]; *Plate Clearing* [39]; *Retrospective Experience of Appetite* (aVAS). Three 24-hour recalls will be completed at four points; before starting the WLP, at the end of the WLP, at the end of the assisted WMP and at the end of the trial. The sFFQ will also be used throughout the WLP and the assisted WMP and at the end of the trial (end of non-assisted WMP) as a means of assessing whether beverage condition influences sweet food intake. Additional cognitive tasks will also be performed and include the *Leeds Forced Choice Test* and the *Leeds Food Preference Questionnaire* [40] assessing the desire for a range of sweet and savoury high and low fat items. Expected satiation of beverages will be assessed using a computerized task as developed by Martin et al. [9]. Visual analogue scale (VAS) questionnaire will be used to assess expected liking, actual liking and actual satiation of NNS beverages or water (see Table 1 for a schematic representation of study visits).

For experience of the WLP and WMP: *Experience of Programme* (eVAS), *Entering Treatment Self-Regulation Questionnaire* (ETSRQ), *Continuing Treatment Self-Regulation Questionnaire* (CTSRQ) and *Diet Self-Regulation Questionnaire* (DSRQ) measuring motivation to participate in treatment and how much of this is controlled (perceived pressure) or autonomous (personal reasons).

For mood and well-being: *Profile of Mood States* (POMS; [41]) assessing mood disturbances; *Perceived Weight Status* [42]; *Everyday Weight Discrimination Scale* [43]; *Exercise Avoidance Scale* [42]; *Self-Esteem Scale* [45]; *Short Warwick-Edinburgh Mental Well-being Scale* [46]; *Satisfaction with Life Scale* [47],and the *Retrospective Experience of Mood* (mVAS).

Exercise measurements

Activity monitors (Fitbit) will be supplied to all participants and will be worn every day for one week during relevant periods. Specifically, they will be provided at baseline and at the end of

each phase of the trial (WLP, assisted WMP and non-assisted WMP). The wristbands consist of a simple band strapped around the wrist which displays and tracks total step count, total minutes of physical activity (separated into light, moderate and vigorous), total minutes sedentary, and total calories burned. Display screens will be covered to ensure participants cannot track their progress. Participants will be provided with instructions of use and will be asked to wear the device continuously other than to recharge the device, when in prolonged contact with water (more than 20 minutes), or when sleeping.

Biochemical measurements

Fasting blood samples will be collected from all willing participants (participants will not be excluded on the basis of not consenting to supply samples) at baseline and at the end of each phase - WLP, assisted WMP and non-assisted WMP (4 visits in total). They will be analysed for HbA1c, fasting glucose, fasting insulin, lipid profiles (total cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL)) and liver function tests (ALT, AST, GGT). The blood samples will be drawn from the antecubital vein with the participant in a fasting state (10 hour fast with only water consumption permitted) and after at least 5 min rest by trained research staff. Samples will be sent daily to the Royal Liverpool University Hospital where analysis will take place in accredited biochemical laboratories.

Screening Visit

The study protocol will be fully explained to participants (including information about Subsets 1 and 2). Clinical measures will be assessed after consent forms have been signed. Participants will then complete a range of questionnaires assessing abnormal eating behaviour, their medical history, liking for and use of NNS beverages and possible food allergies. Upon successfully passing the screening visit, subsequent study visits will be arranged.

Individual Visits

Participants will complete the full range of psychological measurements at individual visits before onset of the WLP, before the assisted WMP, at the end of assisted WMP and at the end of the non-assisted WMP. Participants will be fasted (10 hour fast), will provide blood samples (if they have consented to this aspect of the trial) and will then be provided with a breakfast before completing the questionnaire and cognitive measures.

Weight Loss Phase (WLP)

The 12-week weight loss phase (WLP) will be based on a dietitian-designed nutritional knowledge and group work programme incorporating a social cognition-based approach (The Colorado Weigh) used by Peters *et al*., [14, 15]. This employs a group based approach (6-20 participants) with frequent meetings (one per week) for a one hour session with a nutritionist-trained researcher who will supervise each session and who will provide materials for participants to complete before their next session. Weight will be measured privately at the start of group sessions with each participant in a separate area. Anthropometrics (waist and hip measures) will be taken every four weeks throughout the WLP and blood pressure readings will be taken at baseline, at the end of the WLP, at the end of the aWLP and at the end of the trial. Participants will begin to log their daily beverage intake (which will continue over entire trial duration) online as well as their exercise (only completed during the WLP and assisted WMP).

Assisted Weight Maintenance Phase (WMP)

The assisted WMP will be conducted in the same groups of participants (6-20) utilising the Colorado Weigh programme. Sessions will be conducted once per month over this 40-week period. Body weight and anthropometrics (waist and hip measures) will be measured privately at the start of each session and an additional blood pressure reading will be taken at the last session.

12-Month Non-Assisted Maintenance Phase and Final Visit

After the final assisted WMP visit, participants will continue to receive either NNS or water beverages for 12 months during the non-assisted WM period. They will be asked to complete the Retrospective Experience of Appetite questionnaire (aVAS), Experience of the Programme (eVAS), and Retrospective Mood Questionnaire (mVAS) at monthly intervals. They will also continue to complete daily beverage and exercise logs online over the duration of the 12-month period.

Appetite Probe Day Sessions (Subset 1)

A voluntary subset of randomly selected participants (n=116; 58 NNS and 58 water) will be invited to additionally participate in the appetite probe day sessions to assess changes in appetite response to energetic (SSB) and non-energetic (water and NNS) beverages. These probe days will be measured at baseline, at the start and end of the WLP, at the end of the assisted WMP and at the end of the non-assisted WMP (15 visits in total). Although the start

of WLP probe sessions will be recently after the baseline sessions, the mind set of participants is anticipated to be different due to the structured WLP sessions being received, thus capturing changes in food choice and energy intake at this early stage. At each time point, one probe day will examine the response to a SSB and on the second and third the response to NNS and water, respectively (the order of all three drinks will be counterbalanced across participants and probe days). On these days, participants will consume the SSB, NNS beverage or water and will not be asked to consume their two portions of NNS or water beverage. Participants will be provided with a fixed load breakfast (providing 25% energy from estimated RMR), the midday beverage preload followed one hour later by an *ad libitum* lunch and a further four hours later by an *ad libitum* dinner which they will self-serve. Participants will then be provided with a snack box to consume at will for the remainder of the evening after dinner. They will also complete appetite and sensory VAS ratings throughout the day (including specific beverage appetite VAS questionnaires) and retrospective appetite and craving questionnaires at the end of the day. Energy compensation will be tracked at the *ad libitum* test lunch, evening meal and snack box. Participants will not be provided with any information about the drinks during the probe days; however the sensory characteristics of the beverages may be evident.

DXA Scan Sessions (Subset 2)

In addition to trial participation, Subset 2 (n=50; 25 NNS and 25 water) will be asked to provide a full body DXA scan after an overnight fast at baseline, at the end of the WLP, assisted WMP and at the end of the non-assisted WMP(4 visits in total). The DXA scan will be used to assess changes in body composition during the trial. Body composition will be determined to quantify total and regional (trunk, limb, android and gynoid) fat mass and fat-free mass.

Table 1: Schematic presentation of study visits. DXA=Dual-energy-X-ray Absorptiometry; ET=early termination; FFQ=Food Frequency Questionnaire; N-WMP=Non-Assisted Weight Maintenance Phase, no=number; Sub=Subset, Q=Questionnaire, ET – Early Termination

	Screening					**Weight Loss Phase**																	**Active Weight Maintenance Phase**														**N-WMP**	
Week no	-2	-1	-1	0	0	1	1	2	2	2	3	4	5	6	7	8	9	10	11	11	11	12	12	14	19	24	28	32	36	40	44	48	50	50	51	52	52	53-103	104
Visit no	1	1a	1b	1c	2	3	3a	3b	3c	4	5	6	7	8	9	10	11	12	13	13a	13b	13c	14	15	16	17	18	19	20	21	22	23	24	24a	24b	24c	25	26/ET	27-38	39
Informed consent	x																																							
Inclusion/exclusion criteria	x																																							
Medical history	x																																							
Q: Screening	x																																							
Concomitant meds	x																																							
Adverse events										x				x				X						x		x		x		x		x				x	x			
Group meeting					x	x				x	x	x	x	x	x	x	x	X	x				x		x	x	x	x	x	x	x	x	x								
DXA Scan (Sub 2)					x																		x															x		x
Bloods					x																		x															x		x
Randomization					x																																			
Body weight	x					x				x	x	x	x	x	x	x	x	X	x				x	x		x	x	x	x	x	x	x				x	x		x
Height	x																																							
Blood pressure	x																						x															x		x
Waist & Hip circumference	x					x						x				x							x			x	x	x	x	x	x	x	x	x			x		x
Appetite probe (Sub1)		x	x	x			x	x	x											x	x	x											x	x	x			x	
Q: Probe day Qs (Sub1)		x	x	x			x	x	x											x	x	x											x	x	x			x	
3-d Diet Diary					x																		x													x			x
Sweet FFQ					x																		x													x			
Beverage & Exercise Log						x				x	x	x	x	x	x	x	x	X	x				x		x	x	x	x	x	x	x	x	x				x	x	x	x
Activity armband						x																	x											x						
Product delivery						x					x		x		x		x		x						x	x	x	x	x	x	x	x	x				x		x	
Product drop off										x	x	x	x	x	x	x	x	X	x				x		x	x	x	x	x	x	x	x	x				x		x	
Q: Programme experience										x	x	x	x	x	x	x	x	X	x				x	x	x	x	x	x	x	x	x	x	x				x	x		x
Q: Eating behaviour					x																			x														x	x	x
Q: Attitude beverages					x																			x														x		x

Statistical Analysis

Sample size calculation

The power calculation is based on a similar trial previously conducted [14] using bounds of equivalence which were set to ±1.5 kg for weight loss. Although Peters *et al*. [14] powered for a ±1.7 kg weight loss it is notable that in the EU smaller differences in weight loss between groups may be considered significant. For example, significant and clinically meaningful effects of 1.5kg placebo subtracted weight loss have been sufficient to produce a successful EFSA health claim for weight management [48]. The current trial is powered for a primary outcome of weight loss of 90% power with a two-sided α equal to .05 for bounds of equivalence set to ±1.5 kg for weight loss. The standard deviation for group differences in weight loss (±3.58kg) was derived from Peters *et al*., [14]. Factoring in a 27% attrition rate (average attrition found by Peters *et al*., [14]) it is estimated that 158 participants will be required in each condition (n=316).

A separate power calculation was carried out for Subset 1 (probe day) participants based on one between-subjects condition and five assessment periods and calculated to 90% power and a small to moderate effect size (*f*=.20; G*Power software). The α was set to .01 to represent a partial correction [47] for comparing multiple appetite measures and moderate correlations between the measures were also assumed (r=0.3). This power analysis indicated 58 participants were required in each group (n=116) to power for identification of a significant within-between interaction whilst taking into account a 27% attrition rate.

Primary outcome measures analysis

Changes in mean body weight from baseline to the end of the assisted WMP (end of year one) will be compared using a 2x2 mixed ANCOVA-type model with a between-subjects group factor and within-subjects factor of time and also including baseline weight, age, sex, NNS naiveté and additional covariates as appropriate.

Secondary Analyses

Changes in anthropometrics and body composition (DXA) will be assessed at the same time points as weight change using an ANCOVA-type mixed model with a group-time interaction and also including baseline weight, age, sex, NNS naiveté and additional covariates as appropriate. All questionnaire, cognitive task, biochemical bloods analysis, food diary and exercise data will also be assessed in this way. Group effects as well as other predictive relationships will be explored using multiple linear regression and ANCOVA-type models, including relevant covariates as appropriate. Factor analysis may be employed to construct

latent variables of overarching constructs (for instance individual measures that tap into a single underlying construct, such as craving-type measures will be found) reducing the number of tests run while simultaneously capturing as much variance as possible in underlying constructs. This part of the analysis is data driven and therefore cannot be influenced by experimenter bias. It is notable that condensing measures, through their combination, also limits the issues encountered when applying multiple comparisons. Multiplicity adjustment of p-values will be carried out for pairwise comparisons of groups in the analyses of the primary and secondary outcomes.

In order to investigate the impact of missing data on the primary and secondary analyses, three analysis approaches will be taken. Firstly, a complete-case analysis (CCA) will be carried out. In addition to this, we will also conduct Intention-to-Treat (ITT) analysis which incorporates baseline carried forward (BCF); a last observation carried forward (LOCF) analysis; and an analysis of estimated data conducted using a multiple imputation (MI) analysis. In addition, available-case analyses utilizing all available data will be applied for the exploratory analyses as appropriate.

Discussion

The present trial aims to assess the effects of NNS beverages, as compared to water, on short- and long-term weight management (incorporating weight loss and long-term weight maintenance). It also investigates the underpinning behavioural mechanisms (appetite, energy intake, food choice, mood, attitudes, experiences and changes in preference) that mediate these effects.

Currently, the literature on NNS use in weight gain prevention and weight loss is equivocal, with trial outcomes ranging from NNS use supporting weight loss efforts and diet adherence [14, 15, 16, 18, 50, 51] through a lack of effect on weight gain [11, 12, 17, 28]. Indications that NNS contribute to obesity have been suggested in rodent studies [19, 20, 21, 22, 52]. Thus, further studies are required to elucidate the role of NNS in weight loss and also during long-term weight maintenance, even if the results of some short and long-term interventions appear promising [14, 15].

The present intervention will be two years in length for each participant investigating the effects of NNS beverages compared to water during distinct periods of weight change. This includes three discrete phases: Phase I - weight loss (12-week dedicated weight loss phase with weekly dedicated dietitian-led group sessions), Phase II - assisted weight maintenance (40-week phase with monthly dedicated dietitian-led group sessions) and Phase III - non-assisted weight maintenance (12-month phase of NNS or water consumption). Assessments of body weight will determine changes in overall weight loss or gain and body composition will further explore differences in body fat distribution. The analysis of fasting blood samples will provide a clearer insight into the biological impact of NNS beverages, as compared to water controls, on biomarkers of metabolic health, appetite and satiety.

The proposed research is novel not only due to its extended two year duration but also due to the wealth of psychological measures examining the potential behavioural mechanisms of action underlying weight change. In addition to assessment of body weight and anthropometrics, examination of the experience of and perceived ease of weight management in the two groups during these periods will determine whether NNS or water consumption aid adherence to a weight management programme in those who either regularly consume NNS beverages or are largely naïve to NNS beverages. Furthermore, a systematic comparison of the effects of beverages (NNS, water and SSB) on appetite expression (satiation, satiety, liking and wanting) and energy intake (food choice and energy compensation) in a subset of the study population as well as the examination of changes in

food preferences, cravings and eating behaviours (through food diaries and associated questionnaires) in the full study population will determine whether the effects of NNS on appetite are equivalent to water in aiding successful weight management (over the weight loss and weight maintenance phases). Examination of food choice, food preference, cravings and macronutrient intake will specifically highlight potential detrimental or beneficial changes in preference for sweetness as a result of NNS use.

This research will provide a comprehensive view of the role of NNS beverages in our current environment and the behavioural mechanisms underlying observed effects, particularly the long-term effects of such beverages on weight loss and weight maintenance. To our knowledge, such a detailed assessment of the role of NNS beverages as compared to water has not been conducted and would be an invaluable addition to the existing literature.

Research governance

Ethical approval

The University of Liverpool is the Sponsor of the trial (version 2 dated 16.12.2015). The trial was registered at Clinical Trials (NCT02591134) on 23.10.15. Ethical approval was received from the NRES Ethics Committee North West - Liverpool East on 19.05.2016 (Ethics Ref: 16/NW/0347).

Study sponsor

The University of Liverpool is the study sponsor and all research will be conducted within the University in accordance with the Research Governance Framework. All responsibility regarding the recruitment, running, management and financial arrangements will be through the Institution. Potential risks identified will be reported and managed to ensure research follows Good Clinical Practice (GCP) guidelines to the extent that it is reasonably practicable in a non-medical trial to ensure the highest quality research. Funding for the study is provided by the American Beverage Association.

Data handling and quality assurance

All research undertaken will comply with Good Research Practice guidelines. This involves particular attention to confidentiality and compliance with the Data Protection Act. Personal data will be stored securely at the research site and will only be accessible to the research team. All samples and data will be anonymized before analysis and study data will be stored separately from any personal information with separate anonymized codes. No publications

will contain information that will be able to identify individual participants. All information provided throughout the study will be treated as confidential. Paper copies of the data will be kept in locked cabinets and any computerized data will be stored on password protected computers.

The University of Liverpool will be the custodian of the data and will ensure compliance with the Data Protection Act. Data and all appropriate documentation will be stored for a minimum of 15 years after the completion of the study.

Research dissemination

Data analysis will be conducted according to analysis plans and will be completed within the funded time period for the analyses specified in the current proposal. Results from the study will be summarized and published in peer-reviewed scientific and open access journals and/or presented at scientific conferences. As the funding body, the American Beverage Association (ABA) understands that they will have no influence on data collection, analysis or interpretation or writing of the report. The ABA will however have the opportunity to review the draft manuscript at least four weeks prior to submission and provide input for consideration by the researchers. The principal investigator will ultimately be responsible for publishing the findings. The publication may be delayed up to a maximum period of six months to protect commercially sensitive information, if warranted.

Trial status

Ongoing. The study date deadline is July 2019 or once 432 participants have been recruited and have completed the study (including the 12-month non-assisted WM period).

Competing interests

JCGH, JAH, DC, PC, CH and ER have received funding to their Institution for the current study from The American Beverage Association. JCGH and JAH have also received project funding to their Institution from The Coca-Cola Company and JCGH partial funding for a PhD studentship from The Coca-Cola Company. The other authors declare no conflicts of interest. JCGH has received payment from The Coca-Cola Company for consultancy services.

Author's contributions

JCGH, JAH, DC, PC, CH and ER were responsible for the conception and design of the research. UM drafted the manuscript. JCGH, JAH, DC, PC, CH, ER and UM edited and UM

revised the manuscript. All authors approved the final version. We would like to thank Dr. Amy Ahern and Professor Susan Jebb for their comments on the manuscript.

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