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Study title in PICO format: **Possible effects on healthy young adults of sugar- and artificially sweetened drinks in terms of psychological and metabolic outcomes**

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**Abstract**

*Background.* Various lines of evidence indicate that negative metabolic consequences follow when a person regularly drinks a large quantity of sugar-sweetened beverages (SSBs). What is not known is whether SSBs also produce negative psychological effects, such as impairments in short-term memory and increased impulsiveness. Switching from SSBs to ‘diet’ beverages (non-nutritive sweetened beverages; NSBs) would seem to offer a relatively easy dietary change. However, it has been claimed that this can be counter-productive in at least some circumstances.

*Aims. 1.* To test whether SSB consumption produces impairment on psychological tests, relative to NSB consumption and a water control condition; 2. To test whether the effects of NSB consumption depends on other aspects of a person’s diet (e.g. consumption of sweet energy-rich foods).

*Design.* In a non-blinded 3-group parallel design over a 12-week period the Sugar group is given SSBs, the Diet group is given NSBs and the Water group is given water. Metabolic and cognitive/behavioural measures are taken at the outset of the intervention (Initial test), after 6 weeks (Midway test), at the end of the intervention (Completion test) and approximately 12 weeks later (Follow up test).

*Main predictions:* 1. In the Completion test the primary psychological outcome measure, scores on the Logical Memory test, will be lower in the Sugar group than in the other two groups. 2. In the Completion test the primary metabolic outcome, waist:hip ratio will be larger in the Sugar group than in the other two groups.

**Introduction**

The worldwide increase in consumption of sugar-sweetened beverages (SSBs) such as colas and ‘energy’ drinks has been seen as a major contributor to the obesity epidemic and associated diseases. Intervention studies have confirmed both that decreasing SSB intake can have positive metabolic consequences and that increasing SSB intake can have negative ones; e.g. increases in body weight, total energy intake, and/or triglyceride and cholesterol levels (Miller & Perez, 2014; Rogers, Hogenkamp, de Graaf, et al., submitted). However, none of these studies have included psychological measures.

The possibility that a high level of SSB consumption can affect cognitive functioning is suggested by rodent studies that have found impaired performance on some behavioural tests, particularly those involving spatial learning and memory. In these studies, rats or mice were maintained on diets high in fat and sugar (HFHS diets), on ‘junk’ or ‘supermarket’ diets (e.g. foods typically eaten by people), or even simply given access to a sugar solution in addition to their normal (and healthy) chow diet (Kendig, 2014). Furthermore, Australian undergraduates who report being on HFHS diets do not perform as well on certain neuropsychological tests as those reporting being on healthier diets (Francis & Stevenson, 2011; Brannigan, Stevenson & Francis, 2014). Some authors have suggested that such cognitive impairment contributes to an individual’s overconsumption of food via a vicious cycle, whereby dietary-induced impairment of short-term memory and increased impulsivity leads to further increases in food consumption (Francis & Stevenson, 2011; Davidson, Sample & Swithers, 2014.) However, the evidence supporting such claims is as yet indirect.

For someone on a generally unhealthy diet that includes high consumption of SSBs one kind of dietary change is less of a challenge than most others: this is to switch from SSBs to NSBs. There is good evidence that this can produce a reduction in body weight – though on average a very modest one – and reduce the risk of diabetes. The only prominent dissenters from the general conclusion that SSBs are worse for the body than NSBs have been Swithers and Davidson who base their claims on rat studies (Davidson et al., 2014). According to their argument the relative effects of SSBs and NSBs should depend on whether or not participants’ diets already contain many sweet energy-rich foods; ‘I’ve been drinking diet coke, so it is OK to have another slice of cake’. Thus, Swithers and Davidson predict for people already on a sweet diet that NSBs will be more harmful – at least in terms of weight gain - than SSBs.

**Aims**

1. To test whether consumption of SSBs at a rate of 12 x 375ml (=4.5L) cans per week produces cognitive impairment in young healthy participants, when compared with drinking the same amount of NSBs or when compared to a control condition in which participants are only given water.
2. To test for the body weight and metabolic differences between SSB and NSB consumption found in previous studies and determine whether these depend on a participant’s habitual diet.

**Hypotheses**

1. Performance on cognitive tasks by the Diet and Water groups will improve relative to the Sugar group.

2. Impulsivity in the Diet and Water groups will decline relative to the Sugar group.

3. Preferred concentrations of sucrose will decrease in the Diet and Water groups relative to the Sugar group.

4. In the Diet group weight/waist circumference gain will be greater in those whose normal diet contains a high proportion of sugar-sweetened foods than in those with a low proportion, whereas in the Sugar and Water groups, weight gain will not vary with the proportion of sugar-sweetened foods in their normal diet.

**Design**

In a non-blinded 3-group parallel design the Sugar group is given SSBs, the Diet group is given NSBs and the Water group is given water. Participants will be asked to consume their allocated drinks and avoid drinking anything else, other than water, tea, coffee, milk, fruit or vegetable juice without added sugar or their usual amounts of alcohol. We will attempt to match groups on: 1. BMI; 2. Normal intake of SSBs; 3. Proportion of sweet calorie-rich foods in the normal diet; and 4. Gender. To allow for individual taste preferences, participants in the Sugar and Diet groups will be given a choice among a set of drinks: e.g. Coca Cola vs Sprite vs Pepsi Cola as SSBs, or Coke Zero vs Sprite Lite vs Diet Pepsi as NSBs, where the SSBs all contain approximately 100g of sucrose per litre.

Participants will be asked to consume the drinks for 12 weeks. Following an initial screening session, they will be tested 4 times: 1. Pre-test; 2. Mid-test; 3. Completion test; and 4. Follow-up test 12 weeks after completion.

The study will be terminated if metabolic measures indicate that the addition of 4.5L of SSBs per week to our participants’ diets is more harmful than the consequences reported by Maersk et al (2012) of drinking 7L per week.

**Magnitude of dietary intervention**

Choice of 12 x 375ml (=4.5L) cans per week of either SSBs or NSBs was based on the following considerations. On the one hand, the larger the volume, the more likely it is that important outcomes will be detected. On the other hand, requiring participants to drink very large amounts could lead to a high incidence of non-compliance; furthermore, given evidence that consumption of SSBs has harmful consequences for healthy young adults, as most clearly documented by Maersk et al. (2012) who required participants to drink 1L daily for 6 months, there are strong ethical grounds for not asking participants to drink such a large amount. Thus, the choice of 4.5L per week was a compromise.

**Participants**

We will recruit healthy participants from the student and campus population in the age range of 18-35 years who are already regular drinkers of SSBs (at least 2 L per week, including, for example, fizzy drinks, energy and sports drinks, sweetened fruit juice and iced coffee). These criteria are for both ethical and scientific reasons.

Potential participants will receive a brief telephone interview to assess their suitability for the study: age, gender, any current health issues (and specifically quizzing for eating-related problems and allergies), any past health issues, weight and height, in addition to SSB consumption. Exclusion criteria at this stage are aged < 18 or >35 years, a previous/current eating related disorder, current mental health diagnosis, BMI < 17.5 or > 26, diabetic, food allergies, or other disorder affecting diet/memory (e.g., PCOS, head injury). Inclusion criteria are BMI 17.5 to 25, healthy, aged 18-35.

Participants who appear acceptable on the basis of the initial telephone-based screening are then invited to a screening session, as described below. If initial measures of BMI, glucose metabolism, blood pressure and/or blood biochemistry, as assessed by Dr. Rooney, reveal a serious health risk, a participant will be advised to consult his or her GP and will not be permitted to continue in the study. Where appropriate, Dr.Rooney, as a registered nutritionist and exercise specialist, will provide advice on health management.

All participants will be given dental hygiene advice, plus toothpaste at this initial session.

**Allocation of participants to study arm (groups)**

If an initial phone call indicates that a potential participant meets the above criteria, s/he is invited to a screening session that includes measurement of BMI and blood pressure.  If these and a further health questionnaire fail to reveal any health risk, then s/he is booked for Test session 1 (Baseline). Neither participants nor researchers will know which arm a potential participant will be allocated to until after a decision about eligibility has been made.

Provisional allocation of a participant to an arm will be made on arrival for Test session 1.  Participants will be randomised in blocks of 6 (2 for each group), with the order within each block determined by a random number generator.

This process will be constrained by attempting to match the groups in terms of 4 participant characteristics; in order of priority these are:

1) BMI;

2) Initial report of sugar-sweetened beverage intake, as measured by the Hedricks BEVQ-15;

3) Proportion of sweet energy-rich foods in their normal diet, as measured by the DFS short questionnaire;

4. Gender.

It may well turn out to be impossible to stratify for more than the first two factors.

**Sample size**

In a convenience sample, Francis and Stevenson (2011) found that a self-reported HFHS diet reduced performance on the Logical Memory test relative to a self-reported LFS diet with an effect size of d=0.81. In order for us to have 80% power to detect a significant effect of our dietary intervention of a slightly more conservative effect size of d=.70  and with α=.05 (two-tailed), a sample size of 34 per group is required. Based on Maersk et al’s (2012) dietary intervention, we anticipate an attrition rate of 33%. Therefore, we will aim to recruit and randomise a total of 153 participants to achieve a final sample of 34 per group.

**Payment of participants**

In terms of time spent on the project for each participant, this will consist of: 0.5 h for an initial screening session, 4 test sessions requiring a total of 4.5 h; weekly visits to the lab to return tabs and collect 12 new cans (say, 12 x 1 h, including travel time). This totals 17 hours that could have been spent on more enjoyable activities. Given that these participants are getting a supply of free drinks and health checks, total payment will be $210 for participants who complete every stage, paid in the following instalments: $10 following initial screening, $30 following Test 1, $50 following satisfactory compliance and completion of the Mid-test (Test 2); $60 following continued compliance and completion of the Completion test (Test 3); a final $60 on completion of the Follow-up test (Test 4). This corresponds to approximately $12.40 per hour.

**Procedure**

*Screening session*

In this first session participants will first be given: (1) the Dietary Fat and Sugar Short Questionnaire (DFS-SQ: Francis & Stevenson, 2011) developed for an Australian student population; (2) The HedrickBEVQ-15 for measuring intake of sugar-sweetened drinks; (3) Height and weight for determination of BMI. The groups will be matched in terms of measures 1 and 2, plus BMI, plus gender. (Details of all tests are given in the Appendix below.)

Participants will then be given a BioMed questionnaire that contains items on medical condition, activity level and sleep patterns. If, despite a positive outcome from the previous phone screening, they report poor health (Q.9) or answer ‘Yes’ to any of Medical History questions 13-22 (e.g. diabetes, heart problems, psychoses) they will be excluded from the study. Blood pressure will also be measured; pressure outside the normal range for this age group (18-35 years) will be an additional criterion for exclusion.

Finally, the yellowness of the skin of their palm and inner forearm will be measured using a spectrophotometer; this provides an index of carotenoids and thus of fruit and vegetable consumption (Stephen, Coetzee & Perrett, 2011; Whitehead, Re, Xiao, Ozakinci & Perrett, 2012). As such, it provides an objective measure with which to compare self-reports in the DFS-Q.

*Components common to all test sessions*

Following the initial screening session, acceptable participants will be required to attend four test sessions. In all sessions the following measures will be taken: height and weight for calculating BMI; waist and hip circumference for calculating waist:hip ratio; blood pressure; and total body fat as estimated by Bio-electrical Impedance Analysis (BIA) using a Quantum IV machine. In addition they will be given the following four psychological tests: Logical Memory (LM); Controlled Oral Word Association Test (COWAT); Go/No go test of response inhibition (GNG); and the short version of the Depression Anxiety and Stress Scales (DASS-21).

*Test Session 1 (initial test, immediately before intervention – 90 min)*

For 24 h prior to this session participants will be required to eat only standard meals and abstain from alcohol and excessive exercise and to abstain from eating or drinking anything but water from 11pm the night before. They will be asked to record what they ate in the 24 h prior to this test and to replicate this as closely as possible prior to Test 3 (see below).

Participants are given the following sequence of events:

1. Finger prick on arrival for assessment of blood glucose, triglyceride, uric acid, lactate and cholesterol levels.

2. Psych tests in order: DASS-21; Logical Memory; COWAT; GNG; BIS impulsivity; Recall Logical Memory. (Possibly measure height, weight and waist before Recall test).

3. Sweetness preference test to assess the concentration of sucrose at which the level of sweetness starts to become less pleasant.

4. Initial finger prick sample and then drink 375ml of SSB (i.e. coca cola) within 4 min

5. Further finger prick samples taken at 15, 30, 45 and 60 min and in between these samples the following measures are taken, in this order:

a) height; b) weight; c) Bioelectrical Impedance Analysis (BIA); d) blood pressure; e) waist and hip circumference. Spectrophotometry.

The purpose of the modified form of the standard 2-h oral glucose tolerance test is to measure the metabolic impact of an acute SSB dose on participants. This modification will provide a snapshot of the metabolic impact participants in the Sugar condition will be regularly exposed to throughout the study. Changes in this measure from Test 1 to its repeat in Test 3 will be of particular interest.

Finally, participants will be instructed in how to consume their cans of drinks: each week twelve 375-ml cans (no more than three a day). They will also be reminded that, other than their allocated drink, they should not drink anything other than water, tea, coffee, milk, fruit or vegetable juice without added sugar or their usual amount of alcohol.

*Drink collection and monitoring*

Once a week for the 12-week period participants will attend the lab to collect their drinks. Returning the tabs from their cans will serve as an imperfect check that they have drunk all 12 cans over the previous week. They will be asked to complete the Hedrick BEVQ-15 questionnaire on each visit.

*Test session 2 (midway test i.e. after 6 weeks of drinks – 45 min)*

This will consist of the common components listed above; i.e. BMI, waist-hip ratio and blood pressure, plus the five psychological tests. In addition, they will be given the DFSQ-SQ; this is to test for possible changes in a participant’s normal diet since entering the study.

*Test session 3 (completion test i.e. after 12 weeks of drinks – 75 min)*

The procedure for this session will be almost exactly the same as for Test Session 1. Thus, prior to this session participants will again be required to eat only standard meals and abstain from alcohol and excessive exercise for 24 h and to abstain from eating or drinking anything but water from 11pm the night before.

Following testing in the same sequence as in Test 1, they will be asked two open-ended questions: 1) ‘When you were assigned to your drink condition, e.g. sugar or diet drinks or water, what did you expect the effects might be?’; 2) ‘What, if any, changes have you made to your normal diet in taking part in the study’?

*Test session 4 (follow up test approx. 12 weeks after completion – 60 min)*

This will be the same as Test 2, height, weight, waist and fat measures, followed by the five psychological tests, but with the following additions: 1) repeat of the BIS impulsivity self-report measure; 2) repeat of the DFS-SQ; 3) repeat of the Hedrick BEVQ-15; and 4) repeat of the Bio-Med questionnaire. The spectrophotometry measure of skin yellowness will also be administered. The last four are to assess any change in eating patterns or life style since these were first administered in the screening session over 6 months earlier.

**Compliance criteria**

Compliance for the purpose of reimbursement consists of: 1) collecting their drinks and returning the tabs each week; 2) attending all four test sessions. Any reduction in this will lead to a pro rata reduction in reimbursement.

Criteria for compliance with the intervention and for the purpose of analysis are: 1) consuming at least 10 cans each week; anyone consuming less than that in two consecutive weeks will be removed from the study; 2) meeting the requirement to drink only the permitted and the allocated drinks in at least 10 of the 12 weeks.

**Data analysis**

Analyses will be conducted per protocol on those participants who complete the first three tests. (Since a high drop out rate is expected for the final, ‘follow up’, test, data from this test will be treated separately.) If a particular participant has less than 5% missing data, then he/she will be considered a completer and the missing data will be imputed via multiple imputation. Per protocol analysis was chosen because the study aims to test the effects of the consumption of SSBs and NSBs on psychological and other health outcomes, which requires that the participants actually consume the SSBs and NSABs.

*Primary outcomes:* The primary psychological outcome, Logical Memory, will be analysed at Test 2, Test 3, and Test 4, separately via ANCOVAs, controlling for baseline Logical Memory (i.e. Test 1), baseline BMI, baseline intake of SSBs, baseline proportion of sweet calorie-rich foods, and gender. Pairwise comparisons will be used to test the differences between each of the 3 groups. The primary metabolic outcome, waist:hip ratio, will be analyses in the same manner.

*Secondary outcomes:* All other outcomes will be analysed in the same way, using ANCOVAs controlling for the relevant baseline score and baseline BMI, baseline intake of SSBs, baseline proportion of sweet calorie-rich foods, and gender – as appropriate.

**Investigator obligations**

1. Study data will be stored for a minimum of 15 years in a form that prevents identification of individual participants.
2. The Chief Investigator will be responsible for ensuring that any adverse event is reported to the University of Sydney Human Ethics Committee without delay.
3. The only foreseeable adverse event is that a participant reacts strongly when providing repeated finger prick samples, perhaps to the extent of fainting. Such an event is likely to occur only in Test Session 1, since the procedure will have become routine by Test 3. If this happens, further testing will be terminated and the participant persuaded to remain in a comfortable position within the lab until it is clear that he or she has recovered. The researcher will then discuss with the participant whether he or she wishes to continue in the study. Handling of unforeseeable adverse events will depend upon the nature of the event.
4. If and when a participant is found to be at risk of diabetes on the basis of their fasting blood glucose level, consultation will be arranged with Dr Rooney, a co-investigator who is both a registered dietician and expert on exercise, so that an appropriate management plan can be agreed that is tailored to the particular participant.

**Appendix A: Details of tests and questionnaires**

**A. Dietary Fat and free Sugar Short Questionnaire (DFS)** (Francis & Stevenson, 2013). This 26-item instrument takes only a few minutes to complete. It assesses the extent to which participants’ diets have a high fat and high sugar content and has been recently validated for a young Australian sample comparable to the pool from which we will be obtaining participants. The sugar sub-scale scores will be of particular interest for the present study.

**B. Brief 15-item Beverage Questionnaire (BEVQ-15)** (Hedrick, Savla, Comber et al., 2012). Fifteen questions cover frequency of consuming a whole range of drinks, from milk to energy drinks. As this was developed in the USA, the wording will be modified slightly where needed for Australian participants. Also, a 16th question is added on diet drink usage.

**C. BioMed Questionnaire.** This includes questions on sleep, hippocampal function, physical activity and other aspects of a participant’s daily life as an assessment of general health status. It comprises sections from other well-validated questionnaires; Professor Stevenson developed this questionnaire for the equivalent of the present study population and has used successfully for 4 years at Macquarie University.

**D. Logical Memory (LM)** (Wechsler & Stone, 1987). Participants are asked to read two 1-paragraph stories and then asked to recite back as much as they recall from a story, while tape-recorded. 20-30 min later they are again tape-recorded when asked to recall the stories again. Percent retention from initial to later recall is the main measure. (This test is particularly sensitive to hippocampal function). Different stories are used on the three occasions a participant is tested.

**E. Controlled Oral Word Association Test (COWAT)** (Loonstra, Tarlow & Sellers, 2010). In this test participants are asked to generate as many words as possible in 60 s (a) beginning with a certain letter – usually F, A or S (phonemic test); and then (b) belonging to a certain category, e.g. animal, (semantic test). Different letters and categories are used on the three occasions a participant is tested.

**F. Barratt Impulsivity Scale - Short Form (BIS-SF)** (Spinella, 2007).This contains 15 questions that require participants to describe how frequently they act or think in various situations, e.g. ‘I act on the spur of the moment’ or ‘I concentrate easily’.

**G. Go/No go test of response inhibition (GNG)** (Batterink, Yokum & Stice, 2010). This computer-based test assesses food-specific impulsivity. In 24 trials a picture of a vegetable is shown and participants are instructed to press a button as quickly as possible when an item appears. In 24 interspersed trials a picture of a dessert is shown and participants are instructed *not* to respond. Inter-trial intervals, when a fixation cross is present, vary from 9 to 19s. In a neuroimaging study involving adolescent girls rates of commission errors, i.e. responding on a dessert trial, were found to positively correlate with BMI and negatively correlate with activation of brain areas involved in response inhibition (Batterink et al., 2010).

**H. Sweetness preference test.** Using an ascending/descending staircase method, participants are asked to rate the sweetness and pleasantness of sucrose solutions in tap water that range in concentration from 5% to 15% in 1% steps. The concentration at which an addition of 1% produces a decrease in pleasantness from the previous step (or vice versa) is taken as a participant’s preferred sweetness concentration (‘bliss point’).

**I. Depression Anxiety Stress Scale – Short Form (DASS-21).** (Henry & Crawford, 2005). This questionnaire is a short form of Lovibond and Lovibond’s (1995) 42-item self-report measure of depression, anxiety and stress, a very widely used and well validated instrument for measuring moods. The DASS-21 has been validated on a non-clinical sample of almost 1800 adult members of the general UK population.

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