Research Ethics Committee Protocol Application
Royal Adelaide Hospital

1. Title

Effects of the artificial sweetener, sucralose, on blood pressure, heart rate and superior mesenteric artery blood flow compared to intraduodenal glucose infusion in healthy older subjects.

2. Investigator Details and Qualifications

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3. Purpose and Aims of the Study

To evaluate the effects of the artificial sweetener, sucralose, compared to glucose and saline (control), on blood pressure, heart rate and superior mesenteric artery flow following intraduodenal infusion, in healthy older subjects.

4. Background and Preliminary Studies

Postprandial hypotension (PPH), defined as a fall in systolic blood pressure (BP) of >20mmHg, or a fall from 100mmHg to 90mmHg, within 2 hours of meal ingestion [1], is an important clinical problem which may result in syncope, falls, cerebrovascular accidents and stroke [2, 3]. The elderly and type 2 diabetics are at greatest risk. Despite its clinical importance, current PPH management is suboptimal [4].

Following meal ingestion, blood shifts from the systemic to splanchnic circulation [5]. In healthy subjects with intact baroreflex mechanisms, compensatory mechanisms maintain BP [6], a response inadequate in PPH patients [5]. While PPH mechanisms are incompletely understood, factors including nutrient delivery rate to the small intestine [7-9] hormone release [9], gastric distension [10-13], impaired baroreflex function [4], and splanchnic blood flow [14-16], appear to be involved.

The BP fall is dependent on the rate of nutrient delivery; in healthy older subjects [1] and type 2 diabetic patients [9], the fall is greater when gastric emptying (GE) of glucose is faster. In healthy older subjects, when the stomach is bypassed and glucose infused intraduodenally, the magnitude of the systolic BP fall is greater following glucose infused at 3kcal/min compared to 1kcal/min [7, 17]. In contrast, gastric distension attenuates postprandial BP. In healthy older subjects, when the stomach

is distended by a 'so-called' barostat balloon, the BP fall during intraduodenal (ID) glucose is reduced [18]

The somatostatin analogue, octreotide, which markedly suppresses GI hormone release, attenuates the postprandial BP fall [19] suggesting a role for GI hormones in PPH. Exogenous administration of the incretin hormone, glucagon-like peptide-1 (GLP-1), which is known to slow GE, has been shown to attenuate the postprandial hypotensive response in both healthy elderly subjects [20] and type 2 diabetes patients [20].

Although gut nutrient sensing mechanisms are unclear, stimulation of taste receptor cells, analogous to lingual sweet taste receptors, is believed to play a role [21-24]. Recent studies indicate the presence of G-protein coupled taste receptors, T1R2 and T1R3, and their taste signal transduction partners, the G-protein gustducin and the transient receptor potential ion channel TRPM5, in the mucosa of both the mouse GI tract and the human small intestine [22-25]. *In vitro* studies have shown that the alpha-subunit of gustducin, a-gustducin, and other elements of taste signals (T1R1, T1R2, T1R3 and TRPM5) co-localise with enteroendocrine L cells [23, 24]. Since IV Glucose does affect GLP-1, interactions with these sweet taste receptors may stimulate GLP-1 secretion [24].

Artificial sweeteners are popular alternatives to carbohydrates in the management of obesity and diabetes [26]. Sucralose is a non-caloric sweetener with inconsistent information on its GLP-1 effects. Sucralose stimulates GLP-1 release *in vitro* in a concentration-dependent manner that can be inhibited by the sweet taste receptor inhibitor, lactisole, suggesting a role for sweet taste receptors on GLP-1 secretion [24]. However, in healthy young subjects, oral sucralose was not shown to stimulate GLP-1 release [27].

The effects of artificial sweeteners on postprandial BP, HR and splanchnic blood flow are currently unknown.

The primary **hypotheses** underlying the current study are that (i) compared to saline, ID infusions of glucose and sucralose will induce a larger reduction in BP and increases in both HR and SMA flow and (ii) ID glucose will have a greater hypotensive response than sucralose.

5. Participants

12 healthy subjects aged 65–80 years, recruited from existing databases and via advertisements placed at the Royal Adelaide Hospital.

Subject selection criteria

Inclusion criteria

- Male or female subjects aged 65–80 years
- Body Mass Index 19-30 kg/m²

Exclusion criteria

- History of diabetes mellitus (or fasting plasma glucose >7.0 mmol/L), severe respiratory, cardiovascular, hepatic and/or renal disease (creatinine clearance <50 mL/min), chronic alcohol abuse or epilepsy or if iron stores, or liver function tests are outside the following ranges:

Alanine aminotransferase (ALT)	< 55 U/L
Alkaline phosphatase (ALP)	30 - 110 U/L
Aspartate transaminase (AST)	< 45 U/L
Total bilirubin	2 - 24 µmol/L
Haemoglobin	115 – 165 g/L (Females)
	130 – 180 g/L (Males)
Ferritin	15 – 200 µg/L (Females)
	30 – 300 µg/L (Males)

- Medication that influence BP or GI function
- History of GI disease, including known gastroparesis, significant upper GI symptoms and gastric surgery
- Smoking >10 cigarettes/day
- Alcohol consumption > 20 g/day
- Severe nasal septum deviation
- Blood donation in the previous 12 week

Withdrawal criteria

- Subjects who experience severe PPH symptoms, e.g. fainting and falls

6. Study Plan and Design

Subjects will attend a screening visit prior to enrolment for a blood test, in which 10 ml of blood will be taken to assess renal and liver function, fasting glucose and iron studies. If the selection criteria are

met, subjects will be enrolled in the study and present to the Discipline of Medicine, Royal Adelaide Hospital on three separate occasions (after fasting from solids for 14 h and liquids for 12 h) at least three days apart. Smoking will be prohibited for 12 h prior to, and on study days [7-9, 17, 28-38].

On each study day, subjects will undergo measurements of BP, HR, SMA blood flow by Doppler ultrasonography [38], CO, SV, blood glucose, serum insulin, plasma GLP-1, GIP and catecholamines under randomised, double-blind conditions [17] (*Figure 1*). Each study will be separated by at least 5 days.

On arrival, a silicone-rubber ID catheter with a duodenal infusion port will be introduced into the stomach via an anaesthetised nostril [7, 11, 28, 31, 35, 36, 38] and its location monitored by measurement of the transmucosal potential difference (TMPD) across the stomach and duodenum using an intravenous (IV) cannula filled with sterile saline in the forearm as a reference [7, 11, 28, 31, 35-37, 39]. A cannula will be inserted into an antecubital vein for blood sampling and an automated BP cuff (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) placed around the opposite arm for BP measurements (systolic, diastolic, and HR)[7, 9, 11, 28, 30, 32-36, 38, 39]. Subjects will have a finger attached to a Finometer Pro (Finapress Medical Systems, Amsterdam, The Netherlands) for continuous measurement of CO, SV, peripheral vascular resistance (PVR) and baroreflex sensitivity [40].

Subjects will receive an ID infusion of either (1) 25 %w/v glucose at a rate of 3 kcal/min, (2) 4 mM sucralose in 0.9 %w/v saline, or (3) 0.9 %w/v saline all in a volume of 300 mL over 60 minutes (i.e. 5mL/min from t = 0 – 60 min). Saline (0.9%w/v) will be infused at a rate of 5mL/min for a further 60min (i.e. t = 60 – 120min) [7, 11, 28, 35, 36, 38, 39].

Following a stabilization period of at least 15 min, BP and HR will be measured at 3 min intervals for 9 min prior to infusion commencement, the average of these measurements representing the 'baseline', and then continuously at 3min intervals from t = 0 - 120 min [7-9, 11, 28, 30, 32-36, 39]. Measurements of CO, SV, PVR, baroreflex sensitivity with the Finometer Pro will occur continuously from t =- 9 - 120 min.

SMA blood flow will be measured before ID infusion, i.e. at t = -3 min, and then every 15 min between t = 0 – 120 min[38] using a Logiq™ e ultrasound system with a 3.5 C broad spectrum 2.5 – 4 MHz convex linear array transducer (GE Healthcare Technologies, Sydney, NSW, Australia).

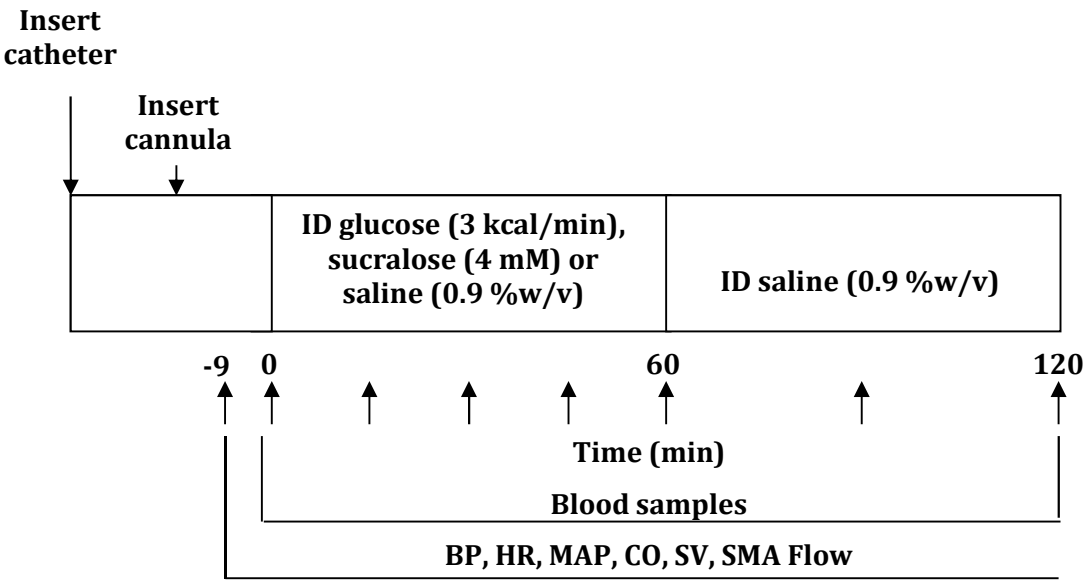

Figure 1: Study design

Venous blood samples (15 mL) will be collected prior to infusion (t = -3 min) and at t = 15, 30, 45, 60, 90 and 120 min for measurement of blood glucose, plasma insulin, total GLP-1, total GIP and catecholamines [3, 7-9, 12, 17, 18, 28-30, 32, 38, 39]. Samples will be stored at -70°C until analysed. Blood glucose concentrations will be immediately determined using a portable blood glucose meter (MediSense Companion 2 meter; MediSense Inc., Waltham, MA) [7, 9, 28, 38].

A visual analogue scale questionnaire will evaluate appetite and other GI symptoms prior to the ID infusion (t = -3 min) and every 15 min between t = 0 - 120min [3, 8, 9, 30].

At t = 120 min, the catheter will be removed, subjects will be offered a light lunch and a final blood glucose and BP measurement will be taken. Subjects will be permitted to leave when their BP returns to baseline levels.

7. Outcomes

The methods to be used are sensitive and well established. The proposed methods are the best currently available to examine the hypothesis described [1, 3, 7-9, 11, 12, 17, 18, 20, 27-39, 41].

8. Ethical Considerations

All aspects of the study will be discussed with each subject during a phone interview or screening visit. An information sheet will be provided, and each participant will be given the opportunity to seek

medical advice or to discuss the study with friends or family prior to involvement. Each participant will give written, informed consent, in accordance with the attached form, and the subjects will be free to withdraw from the study at any time. This study will be performed in accordance with the Declaration of Helsinki.

9. Specific Safety Considerations

Previous studies using the proposed methods [1, 3, 7-9, 11, 12, 17, 18, 20, 27-39, 41], including intraduodenal infusions [7, 11, 12, 17, 18, 28, 31, 37, 38, 41], have been used extensively by our group. The proposed infusion rates have not been associated with any significant adverse effects [7, 11, 12, 17, 18, 28, 31, 37, 38, 41].

Splanchnic blood flow will be assessed by measurement of SMA blood flow using Doppler ultrasonography [12, 38]. Doppler ultrasonography has been used previously to assess SMA blood flow by the investigators [12, 38]. SMA blood flow measurements will be performed by Professor Trygve Hasuken (Bergen, Norway), arguably the world leader in gastric ultrasound. Measurement of SMA blood flow by Doppler ultrasonography is not associated with any adverse effects [12, 38].

Placement of the intravenous cannulae and the silicone-rubber catheter into the stomach via a nostril may be associated with some minor, and temporary discomfort. Bruising, and in rare and extreme cases, infection, may also occur due to the insertion of the catheter.

The Chairman of the Research Ethics Committee will be notified within 72 hours in the event of a serious adverse event.

10. Drugs/Devices

Nil.

11. Analysis and Reporting of Results

Data will be analysed using standardised, non-parametric statistical methods (e.g. using repeated measures ANOVA). Relationships between variables will be assessed by linear regression analysis. The data will be prepared for publication in a peer-reviewed journal. All records will be kept a minimum of 15 years in the Discipline of Medicine and the study will maintain the anonymity of the participants. No medical records will be required for this project. Only the investigators will have access to the research data and results. The Discipline of Medicine, University of Adelaide, will own all data from this study

12. References

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13. Other Relevant Information

Nil.

14. Other Ethics Committees to which the Protocol has been Submitted

Nil.

15. Date of Proposed Commencement

March 2016.

16. Resource Considerations

The project will involve staff, facilities and equipment belonging to the Discipline of Medicine. The project will be supported by funds derived from the Royal Adelaide Hospital Clinical Project Grant. Participants will be offered an honorarium of $18 per hour for time spent in the laboratory.

17. Financial and Insurance Issues

The University of Adelaide will provide indemnification cover.

18. Signatures of Investigators and Departmental Approval

The Principal Investigator (Professor Karen Jones) confirms that the protocol has been read and endorsed and signatures of all investigators have been included in the covering letter as required.

This protocol will be performed in the Discipline of Medicine, University of Adelaide, Royal Adelaide Hospital.