Research Ethics Committee Protocol Application
Royal Adelaide Hospital

1. Title

Effects of a guar and whey containing preload (Omniblend) on gastric emptying and blood pressure responses to oral glucose in healthy older subjects.

2. Investigator Details and Qualifications

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Abbreviations:
BP = blood pressure; GIP= glucose-dependent insulinotropic polypeptide; GLP-1 = glucagon like peptide-1; HR = heart rate; PPH = postprandial hypotension; SMA = superior mesenteric artery; T2DM= type 2 diabetes mellitus

3. Purpose and Aims of the Study

The proposed study is designed to (i) determine the effects of a whey protein/guar preload on gastric emptying and postprandial blood pressure (BP) in response to oral glucose in healthy older subjects and (ii) to determine the relationship between the rate of gastric emptying and the magnitude of the fall in BP following an oral glucose load with and without the preload.

4. Background and Preliminary Studies

Postprandial hypotension (PPH) is now recognised as a frequent and important clinical problem, particularly in the healthy elderly (13 – 38%) [1, 2], and in patients with autonomic dysfunction, often secondary to diabetes (□40%) [1, 3] and Parkinson's disease (40 – 100%) [1, 3]. The consequences of PPH include syncope and falls [3, 4]. More importantly, PPH is also associated with an increase in mortality [5].

The pathophysiology mediating PPH is complex and yet to be completely understood. However, the interaction between autonomic and neural mechanisms plays an important role in the regulation of postprandial BP, including the release of gastrointestinal hormones, which are influenced by meal composition, extent of gastric distension, and small intestinal nutrient delivery. After a meal, there is an approximate doubling of superior mesenteric artery (SMA) blood flow [3]. In healthy young individuals with intact baroreflex function, the increase in splanchnic blood flow is accompanied by concomitant increases in heart rate (HR), as well as peripheral vascular resistance, stroke volume and cardiac output [6]. In patients with PPH, these compensatory responses are inadequate [3]. We have shown that the postprandial fall in BP is greater when gastric emptying is more rapid in patients with type 2 diabetes (T2DM) [7] as well as in a cohort of 86 healthy older subjects [2].

Current strategies for the management of PPH are limited. We have previously demonstrated that gastric distension attenuates the fall in blood pressure induced by intraduodenal glucose in healthy older subjects. More recently, approaches based on slowing gastric emptying [8, 9] and small intestinal absorption of nutrients [10] have attenuated the magnitude of the fall in postprandial BP.

Guar gum, a gel-forming, unpalatable, and unabsorbable carbohydrate, derived primarily from the ground endosperm of guar beans and used as a bulking agent. attenuated the fall in postprandial BP in healthy older subjects [8] and in patients with T2DM [11] when administered together with a 50-g glucose drink. The effects of guar on BP might be attributed to a number of gastrointestinal mechanisms, including slowing of gastric emptying by increasing the viscosity of the intragastric content, as well as delaying small intestinal nutrient absorption by forming a physical barrier between glucose and small intestinal mucosal cells [8, 11], an hypothesis reinforced by the observation that an intraduodenal infusion of glucose (3 kcal/min) and guar (4g) resulted in a lesser fall in BP and a greater increase in HR in comparison to a glucose infusion alone in healthy older subjects [9]. The potential use of guar in the management of PPH is limited due to its poor palatability and gastrointestinal adverse effects such as diarrhoea, flatulence.

An understanding of the effects of different nutrients on postprandial BP is important in the nutritional management of PPH. For example, a low dose whey protein preload ingested before the meal can slow gastric emptying [12, 13] by stimulating release of glucagon-like peptide-1 (GLP-1) and cholecystokinin patients with T2DM [12].

Omniblend (*Omniblend Innovation*), has recently been developed to control postprandial glycaemia. Omniblend is now available in pharmacies and comprises 5g guar, 20g (whey) protein and 3g lactose (total 80kcal) in a sachet, which is added to a 'shake and take' cup containing 150ml water yielding a drink that is available in vanilla, chocolate or 'savoury' flavours. In our 12-week double-blind randomised placebo controlled trial, this supplement slowed gastric emptying, attenuated postprandial hyperglycaemia and was well tolerated in subjects with T2DM (n=79) [14].

More recently, our pilot study of 11 healthy older subjects, demonstrated that a preload of Omniblend given 15 min before a 75 g glucose load tends to attenuate the magnitude of the fall in postprandial systolic BP (Figure 1), with a modest lowering in blood glucose and no change in gastric emptying (unpublished data). The study was generally well tolerated and there were no adverse events. A limitation of this pilot study is the lack of randomisation of the order of the intervention (this was a pilot added onto a study that was already being performed). Furthermore, gastric emptying was assessed using a breath test technique, an indirect measure of gastric emptying which relies on small intestinal absorption. Given that guar gum affects small intestinal absorption [15], this is not the ideal technique; gastric emptying would preferentially be measured via the 'gold standard', scintigraphy.

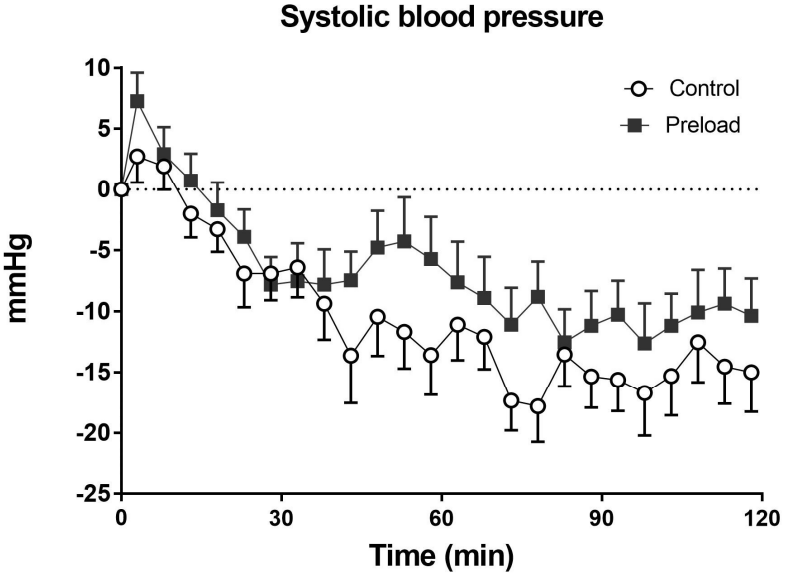

Figure 1: Change in systolic BP from baseline in 11 healthy older subjects following an oral (75g) glucose load with or without an Omniblend preload, administered 15 min before glucose drink (unpublished pilot data).

The primary hypotheses underlying the current study are that (i) a preload of whey protein/guar (Omniblend) will slow gastric emptying in healthy older subjects and (ii) this slowing of gastric emptying will be associated with an attenuation in the fall in BP after oral glucose, and a reduction in splanchnic blood flow. We further hypothesise that the slowing gastric emptying will be associated with a reduction in postprandial hyperglycaemia and glucose absorption will be delayed. The effect of the preload on incretin hormone release (GLP-1 and GIP) is uncertain due to the differing effects of guar and protein on their secretion.

Therefore, the aims of this study are to evaluate the effect of an Omniblend preload on:

i) BP and HR

ii) gastric emptying (using scintigraphy) of a 50g glucose load,

iii) splanchnic blood flow (Doppler ultrasound)

iv) glucose absorption (utilising 3-OMG) and plasma glucose, serum insulin and

v) incretin hormones (plasma GLP-1 and GIP).

5. Participants

18 healthy subjects aged 65–80 years will be recruited from existing databases and via advertisements placed around the Adelaide Medical Precinct including the Royal Adelaide Hospital. Sample size requirements have been determined via power calculations alpha = 0.05 and beta = 0.8, performed with systolic BP as the primary outcome parameter, based on our previous data (*unpublished pilot study*). A sample of 18 subjects are required to determine a difference in systolic BP (postprandial AUC) of 3.2mmHg/min.

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 or HbA1C ≥6.5%)
- Severe respiratory or cardiovascular disease which would limit tolerance of study
- Hepatic disease as defined by transaminases > 2 x upper limit of normal or bilirubin > 2 X upper limit of normal
- Renal disease (creatinine clearance <50 mL/min)
- Anaemia or iron deficiency

Reference 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)

- Medications that influence BP or GI function
- History of GI disease, including known gastroparesis, significant upper GI symptoms or gastric surgery
- Smoking >10 cigarettes/day
- Alcohol consumption > 20 g/day
- Blood donation in the previous 12 weeks
- Exposure to radiation for research purposes within previous 12 months

Withdrawal criteria

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

6. Study Plan and Design

The proposed study is a non-blinded randomised cross-over trial. Due to the nature of the study design, a placebo cannot be provided for the pre-load in this study, as part of the effect of the pre-load is that of stomach distension. The non-preload study condition acts as the 'control' day.

At the first screening visit, patients will have a blood sample (10mL) collected for the assessment of liver function, creatinine, blood picture, iron studies, fasting blood glucose and HbA1c. Cardiovascular autonomic nerve function will be assessed; parasympathetic function, by the variation (R - R interval) of the HR during deep breathing and the immediate HR response to standing ("30:15" ratio) and sympathetic function, by the fall in systolic BP in response to standing [16]. If the selection criteria are met, patients will be enrolled in the study and studied on two separate occasions at least five days apart under randomised conditions. Subjects will present to the Clinical Research Facility on level 4 of the University of Adelaide, Adelaide Health and Medical Sciences (AHMS) Building at approximately 0830h after fasting from solids for 14 h and liquids for 12 h.

Subjects will undergo concurrent measurements of gastrointestinal (GI) symptoms (questionnaire), gastric emptying (scintigraphy), BP, HR (DINAMAP), SMA blood flow (Doppler ultrasound), blood glucose, serum insulin, plasma GLP-1, GIP and 3-OMG [10, 16] (*Figure 2*).

On arrival, the subject will be seated in front of a gamma camera. 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, 17-24].

After a rest-period of 15-30 minutes to allow baseline BP to settle [10], subjects will be given, in random order, either (i) a test drink containing 50 g glucose labelled with 20 MBq ^{99m}Tc-calcium phytate, made up to 300ml water [16] or (ii) a preload containing 20g whey protein and 5g guar (Omniblend) made up to 150ml with water 15 minutes before the ingestion of the test drink. The preload and test drink will be consumed within 2 minutes. Time zero (t = 0) is defined as the time of the end of the test drink ingestion. Dynamic images will be acquired at 1 minute per frame for the first hour and at 3 minutes per frame for another two hours.

BP and HR will be measured at 3 min intervals prior to administration of test drink, and then regularly at 5 min intervals from t = 5 - 180 min [7-9, 11, 17-24]. Baseline BP and HR will be calculated as an average of the measurements obtained at t = -24, -21, -18 minutes prior to the ingestion of the preload (t = -15 minutes), or at t = -9, -6, -3 minutes prior to the ingestion of the test drink (t = 0 minute) on the study day without a preload.

Venous blood samples (15 mL) will be collected prior to the ingestion of the preload (t = -18 minutes) and/or test drink (t= -2 minutes) and then at t = 15, 30, 45, 60, 90, 120, 150, 180 minutes for measurement of blood glucose, serum insulin, 3-OMG, plasma GLP-1 and GIP. 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, USA) and, plasma glucose, subsequently by YSI to reduce the variability observed with the use of a glucometer [7, 17-19].

Splanchnic blood flow will be assessed by measuring SMA flow with Doppler ultrasound [16]. using a Logiq^{TM}9 ultrasound system (GE Healthcare Technologies, Sydney, NSW, Australia). SMA blood flow will be measured immediately prior to administration of the preload (t = -18 min) and/or test drink (t= -2 minutes) and then at t = 15, 30, 45, 60, 90, 120, 150, 180 minutes.

Sensations relating to appetite and PPH symptoms will be evaluated using a visual analogue scale (VAS) immediately prior to the ingestion of the preload (t = -18 minutes) and/or test drink (t= -2 minutes) then at t = 15, 30, 45, 60, 90, 120, 150 and 180 minutes [16].

At t = 180 min, subjects will be offered a light lunch and BP measurements will be taken. They will be permitted to leave when their BP returns to baseline levels.

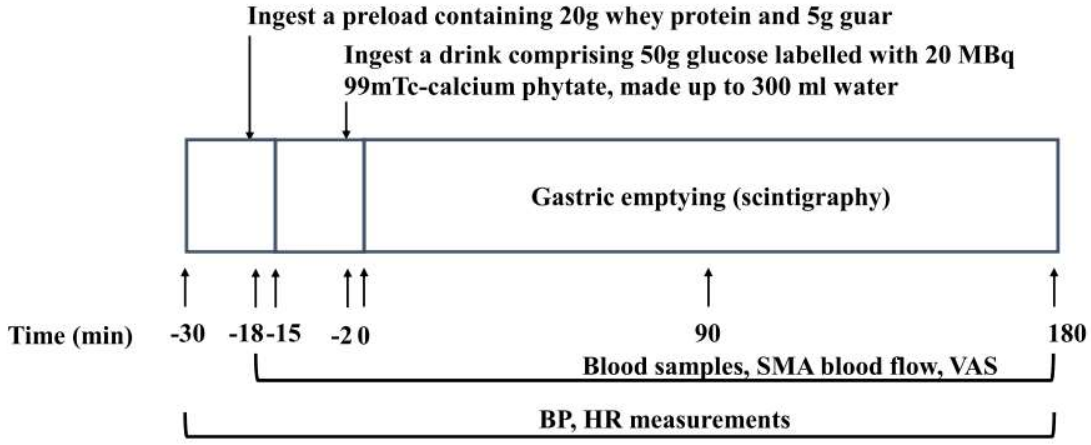

Figure 2: Study design

7. Outcomes

The primary outcomes will be the effects of guar/whey containing preload on gastric emptying, and BP responses to oral glucose as well as the relationships between them in healthy older subjects. Secondary outcomes include effects of guar/whey on: i) SMA blood flow (Doppler ultrasound) ii) postprandial glycaemia (glucose absorption employing 3-OMG, blood glucose and plasma insulin) and iii) incretin effect (GLP-1 and GIP). If the preload attenuates the fall in BP, further evaluation,

including effects of chronic (~4-8 weeks) administration and in patients with PPH, would be indicated.

8. Ethical Considerations

All aspects of the study will be discussed with each subject during a phone interview and 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 Consideration

All techniques are safe and have been extensively employed in published studies conducted by the investigators.

The dose of radiation from each gastric emptying study is within accepted NHMRC guidelines and has been considered acceptable by the Royal Adelaide Hospital Research Ethics Committee (RAH Protocol no.091227B). No women of child-bearing age will be studied with this protocol. Our radiation hot lab, located immediately next door to the Gamma Camera Suite, has been inspected and approved by the Environmental Protection Agency (EPA) as a licenced premise. Professor Karen Jones holds a current radiation licence and is a registered medical radiation practitioner with AHPRA.

Placement of the intravenous cannula 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.

In the case that a patient faints while in the Clinical Research Facility, they will be placed in the supine position with legs raised and attended to by a medical member of staff as is our usual practice.

If a medical condition is identified during the course of this study (eg screening examination, blood tests, or during the study days), one of the physicians associated with the study (Dr Liza Phillips, Professor Chris Rayner or Professor Michael Horowitz) will speak with the subject and liaise with their GP regarding ongoing management/follow up.

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

10. Drugs

Nil

11. Analysis and Reporting of Results

Data will be analysed using standardised statistical methods (e.g. using repeated measures ANOVA). BP, HR will be assessed as changes from baseline, whereas gastric emptying, SMA blood flow and blood glucose will be analysed as absolute values. The maximum changes from baseline in BP, HR and blood glucose will also be calculated. Areas under the curve (AUCs) will calculated for BP, HR, SMA blood flow and blood glucose using the trapezoidal rule. Changes in each variable over time will be evaluated with 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 AHMS Building, or a secure offsite storage facility (e.g. Recall) 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 School 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

May 2018.

16. Resource Considerations

The project will involve staff, facilities and equipment belonging to the NHMRC Centre of Research Excellence (CRE) in Translating Nutritional Science to Good Health. Radiophramaceuticals will be purchased from the Department of Nuclear Medicine, Positron

Emission Tomography and Bone Densitometry, Royal Adelaide Hospital, as is our standard practice.

17. Financial and Insurance Issues

The project will be financically supported by Omniblend Innovation Pty Ltd. Investigators have no conflicts of interest with respect to the outcome of the study.

Subjects will be offered an honorarium of $20 per hour for time spent in the laboratory. Transportation costs will also be covered.

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.