RESEARCH ETHICS COMMITTEE PROTOCOL APPLICATION

**ROYAL ADELAIDE HOSPITAL**

**1. Title**

Effects of a bitter taste receptor agonist (denatonium benzoate) and antagonist (probenecid), in an enema formulation, on gastrointestinal hormone secretion and appetite in healthy humans.

**2. Investigators and qualifications**

1. ***Principal investigator:*** Dr Tongzhi Wu, MD, PhD, RAH Research Committee Early Career Fellow, University of Adelaide Discipline of Medicine, Level 5 Adelaide Health and Medical Sciences (AHMS) Building, North Terrace. Ph: 8313 6535; Email: tongzhi.wu@adelaide.edu.au;
2. Dr Cong Xie, PhD candidate, University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace. Ph: 8313 6595; Email: c.xie@adelaide.edu.au;
3. Ms Michelle Bound, B Med Rad (Nuc Med), Research Officer, University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace. Ph: 8313 6676; email: michelle.bound@adelaide.edu.au;
4. Ms Jacqueline Grivell, B Med Rad (Nuc Med), Research Officer, University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace. Ph: 8313 6691; Email: jacqueline.grivell@adelaide.edu.au;
5. Professor Karen Jones, PhD, University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace. Ph: 8313 7821; Email: karen.jones@adelaide.edu.au;
6. Professor Michael Horowitz, MBBS, PhD, FRACP, University of Adelaide Discipline of Medicine, Royal Adelaide Hospital. Ph: 7074 2673; Email: michael.horowitz@adelaide.edu.au;
7. Professor Chris Rayner, MBBS, PhD, FRACP, University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace; Ph: 8313 6693, Email: chris.rayner@adelaide.edu.au

Address for correspondence (Dr Tongzhi Wu): University of Adelaide Discipline of Medicine, Level 5 AHMS Building, North Terrace, Adelaide, SA, 5000.

1. **Purpose and Aims of the Study**

The gastrointestinal (GI) tract is a logical target for the management of obesity and type 2 diabetes (T2DM), attested to by the success of bariatric surgery and incretin-based therapies. It has recently been appreciated that the GI tract has the capacity to ‘taste’ intraluminal contents in a similar manner to the tongue. Emerging evidence of preclinical studies suggests that bitter substances in the gut can reduce appetite and slow the emptying of meals from the stomach, by stimulating GI hormone release. The purpose of this study is to determine whether stimulation of intestinal bitter taste receptors (BTRs) by non-caloric BTR agonists induces GI hormone secretion in humans and, if so, whether probenecid acts as an effective BTR antagonist.

Specifically, the study will evaluate the hypothesis that (i) rectal infusion of a BTR agonist, denatonium benzoate (DB), stimulates the secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), and (ii) antagonism of BTR signalling by probenecid attenuates the GLP-1 and PYY responses induced by rectal administration of bitter tastants (i.e. DB and taurocholic acid (TCA, which is known to stimulate GLP-1 and PYY secretion humans and therefore will serve as a positive control).

1. **Background and preliminary studies**

***4.1 Overview of research area***

There is growing recognition that GI function is central to the pathophysiology and rational management of T2DM, which represents one of the biggest challenges facing Australia’s health system. The GI tract stores ingested nutrients in the stomach and regulates their delivery to the small intestine at a controlled rate to optimise digestion and absorption. The interaction of nutrients with the small and large intestine generates feedback that slows gastric emptying (GE), induces satiation, and limits postprandial glycaemic excursions ([1](#_ENREF_1)). The mechanisms underlying nutrient-gut interactions are complex; it has only recently been appreciated that the GI tract can detectintraluminal stimuli in much the same way as the tongue, via activation of specific G-protein-coupled receptors (GPCRs), and that ensuing signalling mechanisms regulate the release of an array of gut hormones (including cholecystokinin (CCK) from the enteroendocrine I-cells, and glucagon-like peptide-1 (GLP-1) and peptide YY (PYY) from the L-cells) that determine GI motility, appetite and blood glucose ([2](#_ENREF_2),[3](#_ENREF_3)).

Emerging evidence from our group (**Fig 1**) and others ([4-7](#_ENREF_4)) has shown that stimulation of *intestinal bitter taste receptors (BTRs)* by non-caloric bitter taste agonists (e.g. denatonium benzoate (DB) – the most potent BTR agonist known) markedly suppresses energy intake in healthy humans, and reduces postprandial glycaemic excursions in rodents, associated with augmented secretion of GI hormones and slowing of GE. These complementary metabolic effects, if validated in people with T2DM, would both open a novel avenue for targeting BTRs to improve the management of diabetes, and have a major impact on the food and pharmaceutical industries.Given that physiological bitter substances, including bile acids and products of digestion (such as amino acids), are abundantly present in the gut after a meal, it is also important to understand the physiological relevance of intestinal bitter taste signalling to metabolic homeostasis.

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**Fig 1.** Effect of intraduodenal DB on energy intake (means ± SE) at an *ad libitum* meal in healthy subjects (n=16; #P<0.01 vs. control, *Wu et al. unpublished*)

***4.2 Validation of the effect of a BRT agonist (DB) and antagonist (probenecid) on GI hormone secretion in humans***

Several lines of evidence support an important role of BTR signalling in mediating GI hormone secretion. First, a range of BTRs are expressed on the enteroendocrine L- and I-cells in both rodents and humans ([7-9](#_ENREF_7)). Moreover, activation of BTRs by BTR agonists (e.g. DB and propylthiouracial (PTU)) induces substantial GLP-1 and PYY secretion from enteroendocrine L-cells in a BTR-dependent manner ([7](#_ENREF_7),[8](#_ENREF_8)). In mice, an intragastric ‘preload' of DB slows GE ([6](#_ENREF_6),[10](#_ENREF_10)), and stimulates GLP-1 and PYY secretion thereby increasing insulin secretion and attenuating the postprandial glycaemic excursion ([7](#_ENREF_7)). While we (**Fig 1**) and others ([5](#_ENREF_5)) have shown that BTR agonists (e.g. DB and quinine) potently suppress energy intake in healthy human subjects, the capacity of BTR agonists to stimulate the secretion of GI hormones, particularly GLP-1 and PYY, has not been well established in humans.

Likewise, insights into the physiological role of BTR signalling in the regulation of GI hormone secretion, appetite and postprandial blood glucose have also been limited, due primarily to the lack of a suitable BTR antagonist for use in humans. Recently, probenecid, which is well recognised as an inhibitor of the organic anion transporters (OATs) and used to clinical advantage to inhibit renal resorption of uric acid or reduce the renal excretion of penicillin, has been shown to block a wide range of BTRs both *in vitro* and *in vivo* ([6](#_ENREF_6),[11-13](#_ENREF_11)). In rodents, a dose of 50mg/kg completely abolishes the slowing of GE induced by DB ([6](#_ENREF_6)). Importantly, oral administration of probenecid (10mM) effectively blocks bitter taste perception in humans ([13](#_ENREF_13)). Furthermore, probenecid blocks BTR signalling without affecting non-gustatory GPCRs, and its inhibitory effect on BTRs is rapid and unrelated to OATs ([13](#_ENREF_13)), so that its effect to block intestinal BTRs appears highly specific. Accordingly, probenecid appears to be a useful tool to elucidate the functional importance of BTR signalling in humans.

Given that the GLP-1- and PYY-releasing L-cells are located predominantly in the distal small intestine, colon and rectum ([14](#_ENREF_14)), and that BTRs are abundantly expressed on the L-cells of human colonic mucosa ([9](#_ENREF_9)), it is logical, in this “proof of concept” study, to investigate whether rectal perfusion of BTR agonists stimulates GLP-1 and PYY secretion in humans and, if so, whether this effect can be antagonised by the BTR antagonist, probenecid. We will use the physiological bile acid, taurocholic acid (TCA), as a positive control, because we ([15](#_ENREF_15)) and others ([16](#_ENREF_16)) have shown that administration of TCA into the rectum (1500mg and 3500mg, dissolved in 20mL 1% carboxymethyl cellulose) stimulates substantial GLP-1 and PYY secretion in humans (**Fig 2**), and bile acids are physiological bitter compounds. **Therefore, we will determine plasma GLP-1 and PYY concentrations in response to rectal infusion of DB (30mg) and TCA (3500mg), with or without probenecid (456mg, yielding a concentration > 10mM), in a 20mL aqueous gel as previously, in healthy human subjects.**



**Fig 2.** Rectal TCA stimulates GLP-1 and PYY dose-dependently in healthy humans (n=10, data are means ± SE; # P < 0.01 vs placebo; *Wu et al. Diabetes Obes Metab 2013 (15)*)

1. **Participants**

Power calculations have been performed in consultation with a professional biostatistician (Ms K Lange) employed by our NHMRC Centre of Research Excellence (CRE) in Translating Nutritional Science into Good Health. Based on data derived from our previous study ([15](#_ENREF_15)), 14 healthy subjects will provide 90% power (at α=0.01, to allow for corrections of 4 subgroup comparisons: DB vs. placebo; TCA vs. placebo; DB vs. DB + probenecid; and TCA vs. TCA + probenecid) to detect a difference of 220pmol/L×min in the 2-hour incremental area under the curve (iAUC) for plasma GLP-1 between the treatments. 16 subjects will be recruited to allow for dropouts.

Healthy participants will be recruited by advertisement in the notice boards of AHMS building and the Royal Adelaide Hospital. A copy of the recruitment flyer is included (*Appendix 1*). All participants will be screened according to the criteria detailed below. An honorarium of $20 per hour will be offered for time spent in the laboratory. All volunteers will provide written, informed consent prior to their enrolment in the study.

*Inclusion criteria*

* Healthy male and females aged 18 – 55 years
* Body mass index (BMI) 19 - 25 kg/m2
* Haemoglobin above the lower limit of the normal range (ie. >135g/L for men and 115g/L for women), and ferritin above the lower limit of normal (ie. >30ng/mL for men and >20mg/mL for women)

*Exclusion criteria*

* Use of any medication that may influence gastrointestinal motor function, body weight or appetite (e.g. antihypertensive drugs, domperidone and cisapride, anticholinergic drugs (e.g. atropine), metoclopramide, erythromycin, hyoscine, orlistat, green tea extracts, Astragalus, St. John's Wort etc.)
* Evidence of drug abuse, consumption of more than 20 g alcohol or 10 cigarettes on a daily basis
* History of gastrointestinal disease, including significant upper or lower gastrointestinal symptoms, pancreatitis, or previous gastrointestinal surgery (other than uncomplicated appendicectomy or cholecystectomy)
* Other significant illness, including epilepsy, cardiovascular or respiratory disease
* Impaired renal or liver function (as assessed by calculated creatinine clearance < 90 mL/min or abnormal liver function tests (> 2 times upper limit of normal range))
* Donation of blood within the previous 3 months
* Participation in any other research studies within the previous 3 months
* Inability to give informed consent
* Female participants who are pregnant or planning for pregnancy, or are lactating
* Vegetarians

*Withdrawal criteria*

* Participants will be withdrawn from the study in the event of intolerance of rectal infusions.
* Participants may choose to withdraw from the study at any time.

Participants will be asked to maintain their usual diet and activity levels throughout the study.

1. **Study plan and design**

Prior to being given a randomisation code, participants will undergo a screening visit, at the Clinical Research Facility (CRF) of University of Adelaide AHMS Building, to evaluate their eligibility. Individuals will have had prior opportunity to read the “Information Sheet” and to discuss their participation with their family and friends, if they wish. Investigators will explain the study protocol, and answer any questions the volunteers may have about what is involved, before the volunteers provide written, informed consent. 10 mL venous blood will be collected for blood picture, iron studies, HbA1c, and liver and kidney function. For premenopausal female participants, a pregnancy test will be conducted.

Following enrolment, each subject will be studied on 5 occasions, separated by at least 7 days, in a double-blind, randomized fashion (facilitated by the Royal Adelaide Hospital Pharmacy as previously). On the evening preceding the study day (~1900h), participants will be given a standardised evening meal (McCain’s frozen beef lasagne (McCain Foods Proprietary Ltd, Victoria, Australia); 2472kJ) to consume with water. Following this meal, participants will be asked to fast from solids and liquids (other than water) until the following morning, when they will attend the CRF of the AHMS building at 0800h.

On each study day, an intravenous cannula will be inserted into a forearm vein for repeated blood sampling. The subject will then be positioned in the left lateral decubitus position, and the 20mL aqueous gel (1% carboxymethyl cellulose) containing DB (30mg), DB (30mg) + probenecid (456mg), TCA (3500mg), TCA (3500mg) + probenecid (456mg), or vehicle only, will be infused into the rectum via a soft catheter during t = 0-2min. Venous blood (10mL each) will be sampled at t = 0, 15, 30, 45, 60, 75, 90 and 120min. Blood glucose levels will be immediately measured at the bedside with a glucometer (Medisense Precision QID, Abbott Laboratories, Bedford, MA, USA). Plasma will be separated from the remainder of each sample and stored at - 80 degree Celsius for subsequent measurement of plasma concentrations of GLP-1, PYY and glucose using established assays. At the same intervals used for blood sampling, appetite and gastrointestinal sensations (including hunger, desire to eat, fullness, nausea, bloating, headache, abdominal pain, rectal discomfort, and urge to pass stool) will be assessed using 100 mm visual analogue scales ([17](#_ENREF_17)). At t = 120 min, an *ad libitum* buffet meal will be provided from which subjects will be free to eat as much as they wish for 30 min (t = 120 to 150min), and from which energy intake will be quantified ([18](#_ENREF_18)). They will then be free to leave the laboratory.

An investigator will telephone the subject the day after each study day to ask whether they have experienced any adverse effects, and specifically will ask about each of the symptoms evaluated during the study.

The total amount of blood drawn during the screening and 5 study visits will be 410 mL, approximating the volume of a standard blood donation.

1. **Outcomes**

Primary endpoints will be differences in the iAUC for plasma GLP-1 between the treatments. Secondary endpoints will be differences in the iAUC for plasma PYY and glucose, energy intake and gastrointestinal sensations between the study visits.

1. **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 subject will be given the opportunity to seek medical advice or to discuss the study with friends or family prior to enrolment. Each volunteer will give written, informed consent, in accordance with the attached form, and participants will be free to withdraw from the study at any time. This study will be performed in accordance with the Declaration of Helsinki and the NHMRC National Statement on Ethical Conduct in Human Research (2007).

1. **Specific safety considerations**

We will ensure that a medically qualified investigator is available to attend at short notice whenever studies are scheduled at the AHMS building, in the event of an adverse event.

All techniques are safe and have been extensively employed in published studies conducted by the investigators, and are best available to address the proposed hypothesis.

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 cannula. Rectal infusions will be given using a syringe and a soft catheter; we previously found this to be well tolerated by healthy volunteers ([15](#_ENREF_15)).

The doses of DB (30mg) (**Fig 1**) and TCA (3500mg) ([15](#_ENREF_15)) have been well tolerated in healthy subjects of our previous studies. A comparable dose of DB (~0.3 mg/kg) has been administered orally in children aged 18 to 47 months, without adverse effect ([19](#_ENREF_19)). TCA is regarded by the FDA as GRAS (generally regarded as safe). Rectal administration of TCA (3500mg) increased the ‘desire to defecate’ scores and resulted in one episode of defecation in 9 out of 10 subjects within 8-80 min (median 30 min), without causing any major adverse events ([15](#_ENREF_15)). The dose of probenecid (456mg) employed is less than the standard dose used in the treatment of gout (500mg once or twice a day), and yields a concentration of 80mM in a 20mL aqueous gel, which exceeds the established effective concentration for blocking BTRs in humans (10mM). In the unlikely event of a severe adverse effect, the subject will be excluded from the study and the Chairman of the Ethics Committee notified within 72 hours of the occurrence.

1. **Drugs**

Sodium taurocholate and probenecid, formulated in an aqueous gel (1% carboxymethyl cellulose).

1. **Analysis and report of results**

Data will be analysed using standardised, non-parametric or parametric statistical methods where appropriate (e.g. repeated measures ANOVA). 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 anonymity of the participants will be maintained.

1. **References:**

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1. **Other relevant information**

None

1. **Other ethics committees to which the protocol has been submitted**

None

**15. Date of proposed commencement**

10 January 2018

**16. Date of expected completion**

20 December 2019

**17. Resource considerations**

All of the resources (staff, equipment, materials, funding) required for the conduct of the study are available in the Discipline of Medicine.

**18. Financial and insurance issues**

The project is funded by a University of Adelaide ECR Development grant and the NHMRC project grant (APP1147333), awarded to the Principal Investigator Dr Tongzhi Wu.

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

Insurance and indemnity for the study will be provided by the University of Adelaide.

1. **Signatures of Investigators**

The Principal Investigator (Dr T Wu) confirms that the protocol has been read and endorsed and the signature has been included in the covering letter as required.