School of Medicine, Dentistry and Nursing

Study Protocol and Statistical Analysis Plan

Impact of Inulin on Production of Phenolic Acids from Tomato Onion and Lovage Soup

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Study Protocol and Statistical Analysis Plan

Study design: Acute human bioavailability studies, with a cross-over design. In some cases, using stable isotope labelled enriched polyphenols added as a spike of enriched parent compound.

Arm/ group information

Non blinded - crossover

1. Soup containing tomatoes, red onion and lovage (polyphenol rich) (TOL)
2. Inulin (Non digestible carbohydrate; NDC) (INU
3. Mixture of soup and inulin (TOL+INU)

Each soup will also contain 1g paracetamol to allow estimation of effects on gastric emptying; breath hydrogen will be collected to determine any effects on mouth to caecum transit time.

Wash out duration between feeding studies: one week

Recruitment: Self-reported healthy adults, 20-70y any gender

Exclusion criteria: antibiotic use within the last 3 months, identified gastro-intestinal diseases, on prescribed medication other than the contraceptive pill, individuals who are pregnant or breastfeeding. Individuals who have been diagnosed as anaemic, as well as those who are allergic to any food, or paracetamol.

Intervention recipes: Polyphenol rich soup containing cherry tomatoes (300g) red onion 100g and lovage 20g. (includes quercetin and rutin), using enriched deuterium labelled onions and lovage grown at the James Hutton Institute in Dundee, UK in some cases.

The NDC (fibre): inulin, was chosen based on previous in-vitro work, at a dosage of 10g per drink.

Background diet: Volunteers will maintain a low polyphenol/ low fibre diet for two days before each study day and until completion of the 24 hour urine collection started on the study day.

Procedures on each study day

Procedure	Occasions	Estimated Time taken	Details
Cannulation and blood collection	3	12-hours	Participants will be fitted with a cannula to facilitate blood sampling. Blood samples will be taken at baseline (6ml). After the start of the intervention, blood will be collected every 15 minutes (6mL) for the first two hours and every 1 hour thereafter up to 8 hours. The cannula will be removed eight hours after the start of the test and the subject will be allowed to leave the unit. The subject will return with a 24-hour urine sample, and at 24 hours a blood sample (6mL) will be collected by venipuncture. Participants may provide a faecal sample at 24 hours.
Feeding study	3	5 min	Participants will be given the test soup (polyphenol rich soup alone, inulin alone, or mixed polyphenol and inulin soup) to eat.
Urine collection after the study	3	24 hours	Participants will be provided with containers for urine collection, which will be collected during the test day and over the next 18 hours split into 0-2 hours, 2-5 hours, 5-10 hours and 10-24 hour collections. Water will be provided ad libitum throughout the day, with encouragements to drink every 30 minutes.
Breath hydrogen monitoring	3	>10	Breath hydrogen will be monitored via expelled breath at regular 30 minutes intervals until a rise in hydrogen has been observed.
Lunch at the unit	3	30 minutes	Participants will be given a low fibre low polyphenol meal. The amount eaten will be recorded.
Palatability testing	3	30 minutes	Participants will be asked to fill a short questionnaire regarding the palatability of the soup within 30 minutes of ingestion.

Statistical analysis Plan

This study will test the hypothesis that combination of polyphenolics and inulin in a soup made with Tomatoes, onion and lovage will increase the phenolic acid urinary output in comparison to the same soup without inulin.

Sample size:

Our previous work with orange juice in healthy sedentary females led to an excretion of 598±144 umoles/24h of phenolic acids in urine. To detect a difference in phenolic acid output by 20%. using a matched pair, one-side test, with power at 80% and alpha at 5%, we require 15 participants in this bioavailability study.

Randomization of participants:

The participants will be randomized to the order of diets using opaque envelopes with test food order enclosed.

Test for normality:

Data will be tested for normality by the Shapiro Wilk Test, if non parametric data will be log transformed before statistical analysis.

Primary outcome:

The primary outcome is total urinary phenolic acid content over 24 hours before and after the test meal (umoles/L, mg/L, umoles/day). The effect of inulin will be tested by repeated measures analysis of variance (ANOVA) followed by post hoc Bonferroni to correct for multiple testing.

Secondary outcomes:

Individual and total phenolic acid content in urine at 0-2hours, 2-5, hours. 5-10 hours and 10-24hours. (umoles/L, mg/L, umoles/day

These will be compared by repeated measured ANOVA and post hoc Bonferroni to correct for multiple testing .

Plasma glucose levels **(mmoles/l)**

This will be measured at baseline before the meal and then every 15 mins for 2 hours then every 1 hour for 6 hours . The data will be compared by repeated two way repeated measures ANOVA as changes over the day and also converted to area under the curve and compared between arms by repeated measures ANOVA.

Plasma insulin **(mIU/ml)**

This will be measured in plasma every 15 mins for 2 hours then every 1 hour for 6 hours . The data will be compared by repeated two way repeated measures ANOVA as changes over the day and also converted to area under the curve and compared between arms by repeated measures ANOVA.

Satiety hormones GLP-1 and PYY (picomoles/l)

These will be measured in plasma every 15 mins for 2 hours then every 1 hour for 6 hours . The data will be compared by repeated two way repeated measures ANOVA as changes over the day

Gastric emptying (T1/2 mins)

Paracetamol levels in plasma will be measured over 240 mins to estimate gastric emptying rate. The data will be analysed as area under the curve. The t1/2 for gastric emptying between arms will be compared by repeated measures ANOVA followed by Bonferroni to correct for multiple testing

Mouth to caecum transit time (hours/ mins)

Breath hydrogen samples will be collected at baseline and every 30 mins. Mouth to caecum transit time in minutes will be estimated as the first sustained rise in breath hydrogen. Difference between arms will be repeated measures ANOVA followed by Bonferroni to correct for multiple testing

Faecal phenolic acids (umoles/g, mg/g)

If sufficient faecal sample has been provided, phenolic acids will be measured. The amounts will be compared by repeated measures ANOVA and Bonferroni correction.