University of Glasgow

School of Medicine, Dentistry and Nursing

Study Protocol and Statistical Analysis Plan

Interactions Between Fibre and Polyphenols on Colonic Phenolic Acid Production and Biomarkers of Health (6 Weeks)

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Study Design: 6 week double-blind feeding trial with three arms in a parallel design.

Arm/ Group:

1. Soup containing tomatoes red onion and lovage (polyphenol rich) (TOL)
2. Soup containing tomatoes red onion and lovage and inulin (TOL+INU)
3. Habitual diet

Recruitment: Self-reported, generally healthy 40-70y any gender, BMI > or = 25 not on antibiotics within 3 weeks of starting of study, no medication likely to influence gut function, colonic bacterial activity

Exclusion: antibiotics, pregnancy, lactation.

Intervention recipe: Polyphenol rich soup containing cherry tomatoes (300g) red onion (100g) and lovage (20g) (This naturally includes quercetin compounds). One soup contains 10g of inulin a non-digestible carbohydrate (NDC, fibre).

Protocol: One portion of soup will be incorporated into the diet every day for 6 weeks.

Samples of fasting blood and 24-hour urine will be taken at baseline after 3 days 'standardised habitual diet and after 3 weeks and 6 weeks. The standardised habitual diet will be repeated for three days before each measurement period. In these last few days the test foods may also include a relatively low enrichment, stable isotope labelled plant product to allow identification of phenolics produced from test polyphenols in urine against the background of the normal diet.

Measurements: Fasting plasma levels of antioxidant potential (FRAP), ghrelin, insulin and glucose (from which we will estimate insulin resistance) glycation of proteins, and inflammatory cytokines. Urine will be collected for 24 hours at each measurement period and will be used to measure phenolic acids by GC MS, urinary proteomics biomarkers of coronary arterial disease and metabolomics fingerprints which have been shown to change after polyphenol rich foods and can be related to disease risk.

Faecal samples will be collected for microbiome composition by metagenomic approaches and phenolic acid and short chain fatty acid content.

Satiety at the beginning and the end of the 6-week intervention over one day after the ingestion of the test food in the morning with visual analogue scales, and ad libitum food intake at a buffet meal. Subjects will record a seven-day weighed intake of their diet at the beginning middle and end of the study to consider impact of the juices on habitual energy intake and choice of foods over the study period and under free living conditions. Body weight and % body fat will be measured by bioimpedance to check for potential impacts of changes in energy intake.

Statistical Analysis Plan

The study will test the hypothesis that combination of polyphenols and inulin in a soup made of tomatoes, onion and lovage will increase phenolic acid urinary output in comparison to the same soup without inulin after a 6 week feeding trial of one portion of soup a day.

Sample size:

30 adults per group is sufficient to see an increase in urinary phenolic acid excretion (489±269 to 739±309uM, allowing for 20% drop-out, alpha 0.05, beta 0.8) after 6-weeks of juice supplementation based on our previous work with purple grape juice. This sample size also allows detection of an effect size of 0.84 between groups, and to detect a difference in mean proteomic CAD score of 0.15 between interventions, assuming a 0.25 a.u standard deviation for CAD.

Randomisation of participants:

The participants will be randomised to the dietary groups following a stratified allocation based on gender and BMI.

Test for normality:

Data will be tested for normality by the Shapiro Wilk Test and if appropriate be log transformed before statistical analysis.

Primary Outcome:

The primary outcome is total urinary phenolic acid content over 24 hours at baseline, 3weeks and 6 weeks. (umoles/l, mg/l umoles/day). The difference between trials will be tested by analysis of variance strategies with correction for multiple testing.

Secondary Outcomes

Individual phenolic acid content in urine at baseline, 3 weeks and 6 weeks: (umoles/l, mg/l umoles/day). The difference between trials will be tested by analysis of variance strategies with correction for multiple testing.

Fasting plasma glucose level (mmoles/ml)

This will be measured at baseline, 3 weeks and 6 weeks. The difference from baseline and between trials will be tested by analysis of variance strategies with correction for multiple testing

Fasting Plasma Insulin (mIU/ml) Insulin resistance (HOMA)

This will be measured at baseline, 3 weeks and 6 weeks. The difference from baseline and between trials will be tested by analysis of variance strategies with correction for multiple testing.

Antioxidant potential (FRAP), ghrelin, inflammatory cytokines, glycated protein.

These will be measured using standard techniques at baseline and 6 weeks. The difference from baseline and between trials will be tested by analysis of variance strategies with correction for multiple testing.

Body Weight (kg) and % body fat

These will be measured at baseline, 3 weeks and 6 weeks. The difference from baseline and between trials will be tested by an analysis of variance strategy with correction for multiple testing.

Satiety and food intake

Participants will be provided with the test soup for breakfast and after 3 hours will choose lunch from a buffet. The amount (g) and types of foods eaten will be measured and used to indicate satiety. This will be measured at baseline, 3 weeks and 6 weeks. The difference from baseline and between trials will be tested by analysis of variance strategies with correction for multiple testing

Participants will also fill in visual analogue scales after the meal to indicate their desire to eat and their feelings of fullness at 30 min intervals. The profiles will be compared by an analysis of variance strategy with correction for multiple testing

7-day weighed food diaries will be completed at baseline, 3 weeks and 6 weeks. Energy and macronutrient intake will be measured and compared by analysis of variance strategies with correction for multiple testing

Proteomics, Metabolomics and Gut microbiome

Urinary proteomic biomarkers of coronary arterial disease (CAD) and metabolomic fingerprints will be compared at baseline and 6 weeks using relevant bioinformatics strategies and correction for multiple testing.