Statistical Analysis Plan

The effects of types of fruits and vegetables on vascular function in prehypertensive participants: a pilot study

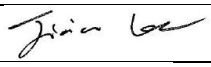
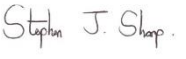
SAP revision history

Date	Version	Justification for SAP version
24 Jan 2022	1.1	Initial draft.
04 Feb 2022	1.2	For review.
04 Mar 2022	1.3	No comments received during review. Version for sign off

SAP responsibilities

Role in SAP development	Name	Role in trial
SAP author	Jian'an Luan	Study statistician
SAP reviewer 1	Stephen Sharp	Overseeing statistician
SAP reviewer 2	Linda Oude Griep	Principal Investigator
SAP reviewer 3	Paul Elliott	Co-Investigator
SAP reviewer 4	Gary Frost	Co-Investigator
SAP reviewer 5	Elaine Holmes	Co-Investigator
SAP reviewer 6	Nick Wareham	Co-Investigator

SAP signatures

Role	Name, affiliation	Date	Signature
Trial PI	Linda Oude Griep	4/3/2022	
SAP author	Jian'an Luan	4/3/2022	
Overseeing statistician	Stephen Sharp	4/3/2022	

1 Introduction

This document describes the primary and secondary analyses of data from a pilot study whose aim is to evaluate the effects of increased intakes of citrus fruits and cruciferous vegetables on vascular function in untreated, prehypertensive participants.

Following completion of the main trial analyses described here, further exploratory analyses of the data from this study may also be conducted. These are not part of this analysis plan.

2 Study design

See Protocol – Section 3.
ClinicalTrials.gov Identifier: NCT03410342

3 Statistical principles

3.1 Confidence intervals and p-values

95% confidence intervals will be reported for the intervention effects on all outcomes. p-values will only be reported for the primary outcomes. Because this is a pilot study, no adjustment for multiple comparisons will be made.

3.2 Adherence and protocol deviations

Adherence to the intervention diets will be analysed using completed daily food checklists.

3.3 Analysis populations

The analysis population will include all participants who have study baseline measures and an outcome measure at the end of at least 1 of the intervention periods.

4 Trial population

4.1 Screening data

See Protocol - Section 3.2.

4.2 Eligibility criteria

See Protocol - Section 4.2 (Table 4).

4.3 Recruitment

See Protocol - Section 4.1.

4.4 Withdrawal/loss to follow-up

The number and percentage of participants who withdraw during the study for any reason will be presented by randomised sequence.

4.5 Baseline characteristics

The following variables measured at baseline will be summarised by randomised sequence. Continuous variables will be summarised using the mean and standard deviation (SD), or median and interquartile range if the distribution is skewed. Categorical variables will be summarised using frequencies and percentages within each category.

- Age (years)
- Sex
- BMI (kg/m^2)
- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)
- Pulse wave velocity (m/s)
- Augmentation index (%)
- Total cholesterol (mmol/l)
- HDL cholesterol (mmol/l)
- LDL cholesterol (mmol/l)
- Triglycerides (mmol/l)
- C-reactive protein (mg/L)

5 Analysis

5.1 Outcome definitions

Primary outcomes:

- Systolic blood pressure (mmHg)
- Diastolic blood pressure (mmHg)

Secondary outcomes:

- Pulse wave velocity (m/s)
- Augmentation index (%)
- Total cholesterol (mmol/l)
- HDL cholesterol (mmol/l)
- LDL cholesterol (mmol/l)
- Triglycerides (mmol/l)
- C-reactive protein (mg/L)
- Potassium (mmol/L)
- Sodium (mmol/L)
- Urea nitrogen (mmol/L)
- Creatinine (mmol/L)
- Memory (mm)
- Processing speed (ms)
- Attention (ms)
- Executive function (mm)
- Self-rated general health (score)
- Subjective mood (score)

- Mental well-being (score)

5.2 Analysis methods

For each outcome variable, the mean and SD of the variable at each study timepoint will be reported, i.e. randomisation (visit 1), start of first intervention period (visit 2), end of first intervention period (visit 3), start of second intervention period (visit 4), end of second intervention period (visit 5), start of third intervention period (visit 6), end of third intervention period (visit 7). The mean and SD of the change in each outcome variable from the start to the end of each intervention period will also be reported.

A linear mixed effects regression model will be used to estimate the effect of each of the 2 dietary interventions with the control group and its confidence interval. Intervention group and period will be included in the model as fixed effects, and the individual identifier as the random effect. The outcome variable in the model will be the relevant measure at the end of each intervention period. This is consistent with CONSORT recommendations[1] that discourage use of change scores, and also with recommendations by Fleiss et al[2] and Willan and Pater[3] that adjustment for baseline values of the outcome should be avoided in crossover trials.

The model assumes that the residuals, conditional on the random effect, are normally distributed. This assumption will be assessed graphically for each outcome, and if not satisfied, an appropriate transformation (e.g. log- or rank-based inverse normal transformation) for that outcome will be considered.

The model assumes that missing values are missing at random.

5.2.1 Sensitivity analysis

A sensitivity analysis on primary outcomes will be conducted by excluding 6 individuals whose randomised sequences were mixed up.

5.3 Safety data

The number and percentage of adverse events (AEs) will be reported by randomised sequence, overall and subdivided into non-serious AEs and serious AEs.

5.4 Statistical software

Analyses will be performed using Stata version 16.1[4].

6 References

1. Dwan K, Li T, Altman DG, Elbourne D. CONSORT 2010 statement: extension to randomised crossover trials. BMJ 2019;366:l4378.

2. Fleiss JL, Wallenstein S, Rosenfeld R. Adjusting for baseline measurements in the two-period crossover study: a cautionary note. Control Clin Trials 1985;6:192-7.

3. Willan AR, Pater JL. Using baseline measurements in the two period crossover clinical trial. Control Clin Trials 1986;7:282-9.

4. StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.