Study Protocol and Statistical Analysis Plan

Study title: The Effects of a Single Dose of Wild Blueberries on Mood and Cognition in Healthy Young Adults: a Randomized, Double-blind, Placebo-controlled, Crossover Study

Date: 27 May 2021

1. Sample Size

A previous study with a similar crossover design found a significant effect of a blueberry drink intervention on mood using a sample size of 21 (Khalid et al. 2017). For the purpose of this study, we decided to include 30 participants – 15 male and 15 female, to be able to study the influence that sex might have on how the intervention affects our outcomes of interest.

2. Recruitment and Screening

Participants will be recruited using opportunity sampling. The study will be advertised online, through email distribution, and with posters across the University of Reading and the local community. Before interested participants are enrolled in the trial, we will invite them to the lab for a screening and familiarization session to determine if they meet the eligibility criteria, conduct a detailed baseline assessment of predictive factors, and give them an opportunity to become familiar with the cognitive task battery so as to reduce the influence of practice effects. Participants will be compensated with £100 ($141) for completing the study.

3. Counterbalancing and Blinding Procedures

The participants will receive both interventions in a counterbalanced manner, i.e. half will be allocated to the order "blubbery → placebo" and the other half will receive the placebo first. The study will be double-blind. A researcher not involved in recruitment or data collection will prepare the intervention drinks and administer them in opaque shaker bottles. Thus, both participants and investigators will be blinded to the treatment assignment throughout the study.

4. Testing Protocol

Each participant will undergo two identical testing sessions at the Nutritional Psychology Lab. On testing days, participants will arrive at the lab after an overnight fast and will consume a standard breakfast of oat porridge. They will have been asked to follow a low-flavonoid diet on the day prior to testing. Next, participants will provide an intravenous blood sample and will complete the baseline cognitive task battery. When finished, they will consume the intervention drink they have been allocated to, prepared by mixing the blueberry or placebo powder with 250 ml water. Allowing 100 min for absorption, we will take another blood sample and will have participants repeat a different version of the same task battery.

5. Statistical Analysis Plan

The present study aims to investigate the acute (2 hours) effects of a wild blueberry intervention on mood and cognition in a population of healthy young adults. On the recommendation of a statistician, all repeated measures data will be analyzed in RStudio using linear mixed-effects models (LMMs). This technique can be used to model variance relating to both fixed parameters such as experimental doses and random parameters such as individual differences between subjects, within multiple layers of the same model. We will adjust the model for baseline variables, such as sex, fruit and vegetable consumption, physical activity level, alcohol use, and systemic inflammation, where the inclusion of these covariates improves the fit of the model. Subjects will be included as a random factor as a way of controlling for non-independence of data from the same subjects. Where possible, an unstructured covariance matrix will be specified for the repeated observations on a subject within the same period. As LMMs do not require balanced data, subjects with missing data points will not be excluded from the analyses.

References

Khalid S, Barfoot KL, May G, Lamport DJ, Reynolds SA, Williams CM. Effects of Acute Blueberry Flavonoids on Mood in Children and Young Adults. Nutrients. 2017 Feb 20;9(2):158. doi: 10.3390/nu9020158.