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Effect of different doses of beetroot juice on cognitive function and cerebral blood flow

Submission date
26/09/2018

Recruitment status
No longer recruiting

Registration date
27/12/2018

Overall study status
Completed

Last Edited
18/01/2023

Condition category
Circulatory System

[_] Prospectively registered
[_] Protocol
[_] Statistical analysis plan
[X] Results
[_] Individual participant data

Plain English summary of protocol

Background and study aims
Nitric oxide (NO) is a small molecule produced in the body which is involved in the control of several functions such as blood pressure (BP), blood clotting, energy metabolism and inflammation. NO is also involved in enhancing cognitive function, which usually declines as we age and with increased body weight. This can happen by increasing blood flow to the brain. This study will ask participants to drink beetroot juice, which contains NO. We will then investigate whether ingestion of beetroot for three months would increase the concentrations of NO in the body and understand the effects on cognitive function and cerebral blood flow in overweight and obese participants.

Who can participate?
Overweight and obese healthy men or women between the ages of 60-75 years

What does the study involve?
Participants will be randomly allocated to 4 groups:
1. One bottle of beetroot juice containing NO to be taken twice per day, in the morning and at night
2. One bottle of beetroot juice containing NO to be taken once per day at night
3. One bottle of beetroot juice containing NO to be taken once every other day at night
4. One bottle of placebo beetroot juice (NO depleted) to be taken once every other day at night
The study involve 6 visits, 4 visits will be at research facility in Newcastle University and 2 visits will be at Brain Performance and Nutrition Research Facility at Northumbria University.
Visit 1: This is screening visit which involve assessment of eligibility using BMI, blood pressure and participants will be trained on the cognitive tasks (around 2.5 hours, Newcastle University)
Visit 2: This is the first study visit, and participants will be asked to be fast for at least 8 hours before the visit. Baseline testing involves measurement of body composition, vascular and pulmonary function and series of cognitive tasks. Participants will be asked to provide blood, urine and saliva samples beside using salivary strip (a tool help measure nitrite in saliva). Several saliva samples will be asked to collect several saliva samples at home. A number of questionnaire will be asked to completed (around 2 hours, Newcastle University)
Visit 3: This is the day after Visit 2 and participants will continue baseline measurements. In this visit we will measure the cerebral blood flow. Only saliva and urine samples will be required in

addition to use salivary strip (around 1 hour, Northumbria University).
Visit 4: This will be 6 weeks from the previous visit. Blood pressure and body composition will be measured. The cognitive tasks administered at baseline will be repeated.
Visit 5: This will be after 6 weeks from previous visit. The measurements described in the Visit 2 will be repeated in the same order.
Visit 6: This will be the day after Visit 5, and the measurements described in the baseline Visit 3 will be repeated in the same order.

What are the possible benefits and risks of participating?
This is a nutritional intervention and there may be some direct benefit for participants. We will measure a number of blood tests, the weight, BMI and BP of the participants which will be of interest to them. There is a minor risk of bruising, bleeding or infection (very rare) when we take blood samples.

Where is the study run from?
Newcastle University Food Research Centre o(UK)

When is the study starting and how long is it expected to run for?
May 2018 to August 2019 (updated 09/07/2019, previously: December 2019)

Who is funding the study?
Royal Embassy of Saudi Arabia Cultural Bureau (UK)

Who is the main contact?
1. Mario Siervo (mario.siervo@ncl.ac.uk)
2. Abrar Babateen (a.m.o.babateen2@ncl.ac.uk)

Contact information

Type(s)
Public

Contact name
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Additional identifiers

EudraCT/CTIS number

IRAS number

ClinicalTrials.gov number

Secondary identifying numbers

Study information

Scientific Title
Long-term effects of different doses of dietary inorganic nitrate on cognition and cerebral blood flow in overweight and obese older subjects

Study objectives
We hypothesise that cognitive functions, specifically executive function, will be improved after a 13-week intervention with dietary nitrate in overweight/obese older individuals. We expect the greater improvement to be seen in the group who are going to take 2 bottles a day (high dose). The improved cognitive abilities will be related to increased blood perfusion in areas of the brain associated with executive functioning (pre-frontal cortex). In addition, dietary nitrate supplementation will be associated with an increased whole-body NO production and decreased blood pressure, oxidative stress and inflammatory biomarkers.

Ethics approval required
Old ethics approval format

Ethics approval(s)
FMS Ethics Committee, 14/05/2018, reference number 1503_1/4477/2018

Study design
Interventional single-blind placebo controlled randomised parallel clinical trial

Primary study design
Interventional

Secondary study design
Randomised parallel trial

Study setting(s)
Community

Study type(s)
Prevention

Participant information sheet
Not available in web format, please use contact details to request a participant information sheet.

Health condition(s) or problem(s) studied
Age-related impairment of cognitive function and cerebral blood flow, cerebral hypo-perfusion

Interventions
This is a single-blind, randomised, placebo controlled clinical trial. Sixty participants are randomly allocated to 4 intervention groups (15 participant per group). Participants will be asked to consume concentrated beetroot juice (70 ml per bottle) in different dosages and frequency, providing different amounts of inorganic nitrate, depending on their group

allocation. Each 70 ml beetroot juice bottle provides approximately 6.8 mmol of nitrate, whereas the nitrate-depleted beetroot juice (i.e., placebo) provides approximately 0.002 mmol of nitrate. One group of participants will take two bottles of beetroot juice per day and they will be asked to consume one bottle in the morning and the other one at night. Participants in the second group will be asked to consume one bottle at night time. Participants in the third and fourth (placebo) group will be asked to consume one bottle of beetroot juice every other day which will be consumed at night time - the third group will consume the same beetroot juice as the previous group (but every other day), whereas the fourth group will consume the nitrate-depleted, placebo juice.

This is a randomised parallel trial including four main phases:
1. Screening and introductory visit: participants will attend the research facility for an initial screening visit, during which they will be screened according to the inclusion/exclusion criteria. Informed written consent will be received and height, weight, blood pressure and waist circumference will be measured. General information including age, occupation and number of years in formal education will be collected. Participants will then be trained on the cognitive tasks, completing three practice trials for each task to familiarize with the test procedure and to reduce the possibility of learned effects at baseline. At the end of the visit, participants will be provided with labelled urine container and will be asked to collect urine at home in the morning in the day of next visit (study 1 visit).
2. Study visit 1 (baseline) at Newcastle University:
Eligible subjects will be invited to attend the NU Food Research Facility at Newcastle University in a fasted condition (at least 8 hours) for baseline testing. In this visit, body composition will be measured. Blood and saliva samples then will be collected to measure the baseline measurement of nitrate/nitrite in plasma and saliva, along with glucose, insulin, oxidative stress and inflammatory biomarkers in plasma. Salivary strips will also be used to measure nitrite concentration. Then, the pulmonary function will be assessed using a portable spirometer. After that, fractional exhaled NO will be measured using a portable, non-invasive, automated device (NIOX VERO). Then, a battery of cognitive tests will be performed. After 15 min rest, we will then measure their resting blood pressure (BP) in duplicate followed by the assessment of vascular function and pulse wave velocity. The participant will be given a small snack (orange juice and option of muffin or muesli bar) and they will be provided with instruction on what to eat on their lunch. Participants will leave the research unit also with resting blood pressure monitoring device, instructions and necessary materials for the collection of saliva samples, consumption of labelled dose of sodium nitrate (4 mg of $Na^{15}NO_3$ in 100 mL nitrate free water), protocol for the collection of pre- and post-dose saliva samples for the measurement of whole-body NO production. Saliva samples will be collected at 17:00 (baseline), 20:00, 21:00, 22:00, 23: 00 and 07:00, 08:00 and 9:00 on the next day. Participants will be asked to drink a low nitrate water during the test period (Buxton water) and a small meal (two muesli bars and a banana) will be provided to be consumed at home at 19:00. The labelled dose will be administered immediately after the baseline sample (17:00). Participants will be asked to come next day to Northumbria University to complete the rest of baseline measurements (CBF measurements). They will be given a urine container to collect urine at the same time in the morning and will be asked to bring it with them when they arrive research centre at Northumbria University next day. They will be asked to complete a food frequency questionnaire and a physical activity questionnaire at home, and to bring them with them next visit.
3. Study visit 2 (baseline continued) at Northumbria University: In this visit, each participant will bring the saliva samples that have been collected and blood pressure monitors. Then, CBF measurement will be performed using qNIRS at rest and stimulated conditions (while participants will be performing some computerised cognitive function tests). After that, they will be randomised to one of four interventions: 1) two bottles of beetroot juice/day (high dose) 2) one bottle/day (medium dose) 3) one bottle every two days (low dose) and 4) placebo

beetroot juice depleted nitrate one bottle every two days (control). They will be asked to collect urine and saliva samples and use NO saliva test strips in the morning for three continue days every four weeks, which will help to measure their compliance. Participants will leave the research unit with the number beetroot juice bottles they will need over the next 6 weeks and necessary materials for the collection of urine and saliva samples. In addition, NO saliva test strips will be provided. Participants will be asked to post urine and saliva samples and strips in provided closed box.
4. Study visit 3 (after 6 weeks) at NU Food Newcastle University: Participants will return to the research facility after 6 weeks to check their health status and compliance. Body weight and resting blood pressure will be measured. They will be asked to perform same cognitive tasks that they did in visit 1. At the end of the visit, they will be provided with the rest of the allocated beetroot juice bottles to be consumed over the next 6 weeks day and necessary materials for urine and saliva collection, as well as, strips. Participants will be asked to post the urine samples in closed box (after two weeks from this visit).
5. Study visit 4 (after 12 weeks) at NU Food Newcastle University: this visit will follow the same format as the first baseline visit.
6. Study visit 5 at Northumbria University: Participants will be asked to come to Northumbria University the day after study visit 4 and bring the saliva samples they collected and the blood pressure monitors. This visit will follow the same format as the second baseline visit.

Intervention Type
Supplement

Primary outcome measure
Acceptability and feasibility of the proposed intervention study in terms of:
1. Whether participants will be able to consume beetroot for 13 weeks
2. Whether participants will be retained in the study
Participants will be provided with a checklist to record and monitor beetroot juice consumption throughout the study, and an exit feedback questionnaire will be completed at the end of the study to obtain detailed information on the acceptability and compliance to the interventions and measurement protocols.

Secondary outcome measures
Changes in the following from the baseline to the end of the study, assessed at the baseline and the end of the study:
1. Cognitive function, assessed using the Computerised Mental Performance Assessment System software (COMPASS, University of Northumbria)
2. Cerebral blood flow, assessed using a frequency domain ‘quantitative’ NIRS system (OxiplexTS Frequency-Domain Near-Infrared Tissue Oximeter, ISS Science)
3. Body composition, assessed using a bioelectrical impedance analyser
4. Resting clinic and home blood pressure, assessed in duplicate using an automated BP monitor (Omron M3)
5. Endothelial function, assessed using Iontophoresis
6. Metabolic biomarkers:
6.1. Nitrite and nitrate, assessed by ozone-based chemiluminescence
6.2. cGMP, assessed using commercially available and validated immunoassays
7. Metabolic risk factors (blood glucose concentration, assessed using commercially available kits
8. The following oxidative stress biomarkers, assessed using commercially available and validated immunoassays:
8.1. 4-Hydroxynonenal
8.2. 3-Nitrotyrosine

9. Inflammatory biomarkers (IL-6), assessed using commercially available and validated immunoassays
10. Endothelial-dependent and -independent microvascular blood flow, assessed by Laser Doppler Iontophoresis (LDI) and pulse wave velocity measured by photo-plethysmography
11. Whole-body NO production, assessed from saliva samples using a stable isotopic method (gas chromatography/mass spectrometry (GC/MS))

Overall study start date
15/05/2018

Completion date
31/08/2019

Eligibility

Key inclusion criteria
1. Healthy
2. BMI 25-40 kg/m²
3. Aged 60-75 years
4. Non-smoker
5. Non-vegetarian

Participant type(s)
Healthy volunteer

Age group
Senior

Sex
Both

Target number of participants
60 participants in 4 groups of 15 participants each

Total final enrolment
62

Key exclusion criteria
1. Current participation in other research clinical studies
2. Systolic blood pressure lower than 115 mmHg and greater than 160 mmHg
3. Diastolic BP lower than 70 mmHg and greater than 100 mmHg
4. Active cancer and any diagnosis of malignant cancer in the last 5 years
5. Excessive alcohol intake
6. Allergy or intolerance to the intervention food
7. Diagnosis of chronic and acute metabolic and inflammatory conditions interfering with the study outcome, including flu, Crohn's disease, rheumatoid arthritis and epilepsy
8. Major surgical operations interfering with the study outcomes
9. Taking any of the following if the dose has been started/changed in the previous 3 months:
9.1. Hormonal therapies (oestrogens, thyroxin, and progesterone)
9.2. Anti-hypertensive drugs (Ca++ channel blockers, beta-blockers, and angiotensin-converting-

enzyme (ACE) inhibitors)
9.3. Statins
9.4. Any other anti-dyslipidaemic agent
9.5. Psychiatric drugs (antidepressants, sedatives, antipsychotics)
Non-prescribed vitamin or other dietary supplements will be stopped during the study.

Date of first enrolment
01/07/2018

Date of final enrolment
30/04/2019

Locations

Countries of recruitment
England

United Kingdom

Study participating centre
NU-Food Research Center
School of Natural and Environmental Sciences, Agriculture Building, King's Road, Newcastle upon Tyne NE1 7RU
Newcastle upon Tyne
United Kingdom
NE1 7RU

Study participating centre
Brain, Performance and Nutrition Research Centre
Northumberland Building, Faculty of Health and Life Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST
Newcastle upon Tyne
United Kingdom
NE1 8ST

Sponsor information

Organisation
Royal Embassy of Saudi Arabia Cultural Bureau

Sponsor details
Royal Embassy of Saudi Arabia
Cultural Bureau in London

630 Chiswick High Road
London
United Kingdom
W4 5RY

Sponsor type
Other

Website
http://uksacb.org/uk-en1313/page/contact-us

Funder(s)

Funder type
Other

Funder Name
Saudi Arabia Cultural Bureau in London

Alternative Name(s)
Royal Embassy of Saudi Arabia Cultural Bureau in London, Royal Embassy of Saudi Arabia - Cultural Bureau in London, Royal Embassy of Saudi Arabia Cultural Bureau, SACB

Funding Body Type
Private sector organisation

Funding Body Subtype
Other non-profit organizations

Location
United Kingdom

Results and Publications

Publication and dissemination plan
Planned publication in a high-impact peer-reviewed journal, expected to be in July 2020

Intention to publish date
31/12/2020

Individual participant data (IPD) sharing plan
The datasets generated during and analysed during the current study will be available upon request from Dr Mario Siervo (mario.siervo@ncl.ac.uk). Electronic datasets (Excel or SPSS files) will be available from 1st January 2021 for a period of 5 years. Data requests will be evaluated by the main investigators of the study and data will be released if proposed use is within the

scope and aims approved by the ethical committees approving the study in the UK. Written consent was obtained from all participants included in the study. Data will be provided in full anonymised form and recipients will be asked to destroy the dataset at the end of the agreed access period of use. Material transfer agreements will have to be formalised between institutions before transferring the datasets.

IPD sharing plan summary

Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article	results	25/04/2020	15/05/2020	Yes	No
Results article		26/02/2021	18/01/2023	Yes	No
Results article		02/03/2022	18/01/2023	Yes	No