ISRCTN59029976 https://doi.org/10.1186/ISRCTN59029976

Combined effects of nitrate-rich beetroot juice and low calorie diet on metabolic and vascular function in overweight middle-aged and older people

Submission date
07/10/2022

Recruitment status
No longer recruiting

Registration date
28/12/2022

Overall study status
Completed

Last Edited
29/12/2023

Condition category
Other

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

Plain English summary of protocol

Background and study aims
The world population is rapidly ageing. Ageing is associated with changes in body composition (i.e., increase in fat mass and decrease in lean body mass) which lead to an increased cardiovascular (heart disease) and metabolic risk. Currently, obesity is on the rise but the evidence of the most effective and safe strategies for the treatment of obesity at this stage of life remains elusive. A major challenge is the loss of muscle mass during weight loss. Moderate weight loss in older people with obesity with diabetes and high blood pressure is still recommended as the first line of treatment, often prescribed with regular exercise sessions. The latter has been demonstrated to minimize muscle loss and therefore associated with greater improvements in cardio-metabolic functions and long-term weight maintenance. However, physical mobility is often an issue in people with obesity and therefore alternative strategies to reduce lean body mass loss are fundamental in obesity research.
Inorganic nitrate supplementation is linked to an increased generation of protective molecules such as nitric oxide (NO) and could provide a strategy to slow muscle loss during old age and prevent loss of muscle mass. The overall aim of the study is to test whether nitrate-rich beetroot juice and caloric restriction (low calorie diet) can provide greater benefits on metabolic and cardiovascular health than caloric restriction alone.

Who can participate?
Participants aged 50 to 75 years, overweight and obese (BMI between 25-40 kg/m²), non-smoking and weight-stable and without other illnesses or medications affecting the study outcomes

What does the study involve?
The study tests the effects of two interventions: 1) caloric restriction plus nitrate-rich beetroot juice and 2) caloric restriction. The eligibility of participants will be checked at a screening visit during which medical history and inclusion criteria will be evaluated. All participants were required to sign an informed consent. If eligible, participants will be randomly allocated to a

specific intervention and they will be invited to return 1 week after to start the study. Participants will be asked to arrive in the morning after fasting and several measurements will be conducted (blood pressure, resting energy expenditure, body composition, physical function and cognitive function). After the measurements participants will be explained the caloric restriction plan they will have to follow for 14 days. If allocated to the nitrate group, they will also receive 14 bottles of nitrate-rich beetroot juice to consume every morning at home. After 14 days, participants return to the research centre and the same measurements will be performed in the same order.

What are the possible benefits and risks of participating?
Participants may benefit from participating in a caloric restriction plan which may improve their cardio-metabolic health. Participants will be asked to drink 70 ml of concentrated beetroot juice per day corresponding to an average supplementation of 300-400 mg of inorganic nitrate per day. This amount of nitrate intake is commonly observed in subjects with a high intake of fruit and vegetables (particularly leafy vegetables) and it is significantly lower than the nitrate intake that may be observed in vegetarian subjects. There is no established health risk associated with either this level of nitrate intake or with beetroot juice supplementation. All measurements performed in the study have limited intrusiveness and health risks.

Where is the study run from?
University of Naples Federico II (Italy)

When is the study starting and how long it is expected to run for?
December 2014 to December 2019

Who is funding the study?
1. University of Naples Federico II (Italy)
2. University of Newcastle (UK)
3. University of Nottingham (UK)

Who is the main contact?
Prof. Mario Siervo, mario.siervo@nottingham.ac.uk

Contact information

Type(s)
Scientific

Contact name
Prof Mario Siervo

ORCID ID
https://orcid.org/0000-0001-5515-0944

Contact details
School of Life Sciences
Division of Physiology, Pharmacology and Neuroscience
University of Nottingham
Queen's Medical Centre
Nottingham
United Kingdom

NG7 2UH
+44 (0)115 8230279
mario.siervo@nottingham.ac.uk

Additional identifiers

Protocol serial number
Version 2, 20/01/2015

Study information

Scientific Title
A pilot randomized controlled study to evaluate the effect of inorganic nitrate supplementation during caloric restriction on energy metabolism, vascular health and cognition in overweight and obese subjects

Study objectives
The project is based on the hypothesis that inorganic nitrate may enhance the effects of caloric restriction on metabolic and vascular functions. This may be mediated by increased NO production, enhanced muscular mitochondrial function, which may improve muscle energetics and minimize the production of reactive oxygen species and inflammation. This may decrease the loss of lean body mass following weight loss and reduce the expected reduction in resting energy expenditure. These benefits are speculated to be systemic and, hence, also improve physical performance and cognition function.

Ethics approval required
Old ethics approval format

Ethics approval(s)
Approved 22/07/2015, Faculty of Medicine Ethics Committee of the University Federico II, Naples (University Federico II, Naples, Via Pansini, 5, 80131, Italy; +39 (0)817473433; segreteria@comitatoeticofedericoiicardarelli.it), ref: 8615

Primary study design
Interventional

Study design
Two-arm open-label parallel randomized clinical trial

Study type(s)
Prevention

Health condition(s) or problem(s) studied
Cardiovascular and metabolic diseases in overweight and obese adults

Interventions
Two-week, two-arm, parallel, unblinded, pilot randomised controlled trial investigating the impact of dietary nitrate along with caloric restriction on the metabolic and vascular function in 36 non-smoking, stable-weight, middle-aged and older (50-75 years) and overweight and obese (BMI range: 25-40 kg/m²) adults. The intervention lasted 2 weeks and consisted of three visits

(screening and assessment, baseline, and end visit). Eligible participants were allocated randomly, using the block-randomisation procedure (five subjects per block) and calculated through RandList, to i) the inorganic nitrate by beetroot juice + caloric restriction (n = 18); or ii) caloric restriction alone (n = 18) groups.

Interventions: Each participant will be invited to start each intervention at the end of the baseline visit and continue for 14 days until they will return to the weight loss clinic for their final visit (15 days). 18 participants will be allocated to the caloric restriction plus beetroot juice and 18 participants to caloric restriction alone. The two interventions are described below.

1. Caloric Restriction: A hypocaloric low-fat diet will be prescribed to each participant. The caloric restriction will be calculated as 40% of total energy requirements and macronutrient composition will be approximately: CHO = 55-60%, FAT= 20-25%, PRO = 15-20%. The amount of high nitrate food (ie. lettuce, rocket, cabbage, spinach, broccoli, etc) will be similar between diets. Participants will be asked to drink the same water during the 2 weeks intervention. Participants will be asked to not change their habitual physical activity level and alcohol and caffeinated drinks consumption during the trial.

2. Inorganic Nitrate Supplementation: Participants will be asked to drink 70 ml of concentrated beetroot juice per day corresponding to an average supplementation of 300-400 mg of inorganic nitrate per day. This amount of nitrate intake is commonly observed in subjects with a high intake of fruit and vegetables (particularly leafy vegetables) and it is significantly lower than the nitrate intake that may be observed in vegetarian subjects. There is no established health risk associated with either this level of nitrate intake or with beetroot juice supplementation. However, at the screening visit participants will be asked whether they have an aversion to beetroot or beetroot juice and they will be excluded if they report problems with the consumption of these products. Subjects will be invited to drink 70 ml of beetroot juice in the morning. They will be provided with a sheet to record the time of the consumption and if they will experience any problems. Participants will be considered not compliant with the intervention if they will miss and/or do not complete two or more supplementation days. Participants will be provided with the specific amount of beetroot juice to be consumed during the 14-day period.

Intervention Type
Supplement

Primary outcome(s)
Resting energy expenditure measured by indirect calorimetry at baseline and at the end of the study (14 days)

Key secondary outcome(s)
Measured at baseline and at the end of the study (14 days):
1. Body composition measured by bioelectrical impedance
2. Resting blood pressure measured by an automated blood pressure device
3. Endothelial function: microcirculation blood flow during post-reactive hyperaemia measured by laser doppler flowmetry
4. Cognitive function measured by Trail Making Test Part A and Part B
5. Physical function assessed by hand grip strength measured by a hand-held dynamometer
6. Laboratory biomarkers (nitrate, nitrite, markers of oxidative stress) measured using standardised laboratory protocols

Completion date
31/12/2019

Eligibility

Key inclusion criteria
1. Males and females aged 50 - 75 years
2. Overweight and obese subjects (BMI range: 25-40 kg/m²)
3. Non-smokers
4. Weight stable

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Mixed

Lower age limit
50 Years

Upper age limit
75 Years

Sex
All

Total final enrolment
29

Key exclusion criteria
1. Current participation in other research clinical studies
2. Vegetarianism (likely to have very high nitrate intake)
3. Weight change more than 3.0 kg in the last 2 months (important influence on systemic metabolism and vascular function)
4. Active cancer and any diagnosis of malignant cancer in the last 5 years (systemic effects on study outcomes)
5. Diagnosis of chronic and acute metabolic and inflammatory conditions interfering with the study outcome (systemic effects on study outcomes). For example, flu, Crohn’s disease, rheumatoid arthritis
6. Previous diagnosis of type 1 or type 2 diabetes treated with insulin (modification of regulation of intermediate metabolism). Type 2 diabetic patients treated with diet only or and oral hypoglycaemic agents with dose stable for at least 3 months will be included in the study
7. Weight loss medications (sibutramine, orlistat, rimonabant) and history of bariatric surgery (weight loss related changes in systemic metabolism)
8. Drugs: corticosteroids, sildenafil, aspirin, NSAIDs, diuretics, antacids, anticoagulants, nitrate-derived agents, anti-cholinergic, (all drugs may have either an effect on NO production or insulin sensitivity via different mechanisms)

9. Subjects on hormonal therapies (oestrogens, thyroxine, progesteron), anti-hypertensive (Ca++ channel blockers, ACE inhibitors, beta-blockers,), statins and any other antidyslipidaemic agent, and psychiatric drugs (antidepressants, sedatives, antipsychotics) will be excluded if the dose has been started/changed in the previous 3 months (make sure that these disorders are under strict control to avoid interference with the study outcomes)
10. Severe haematological disorders (risk for the participant and effects on the study outcomes)
11. Major surgical operations interfering with the study outcomes (systemic effects on study outcomes)
12. Alcohol intake >21 units/week for men and >14 units/week for women
13. Blood donations in the previous 3 months

Date of first enrolment
01/02/2016

Date of final enrolment
03/12/2017

Locations

Countries of recruitment
United Kingdom

England

Italy

Study participating centre
Federico II University of Naples
Department of Clinical Medicine and Surgery
Via S. Pansini, 5
Naples
Italy
80131

Study participating centre
Newcastle University
Human Nutrition Research Centre
Population Health Sciences Institute
Newcastle upon Tyne
United Kingdom
NE2 4HH

Study participating centre
University of Nottingham Medical School
Queens Medical Centre

Derby Road
University Park
Nottingham
United Kingdom
NG7 2UH

Sponsor information

Organisation
University of Naples Federico II

ROR
https://ror.org/05290cv24

Funder(s)

Funder type
University/education

Funder Name
Università degli Studi di Napoli Federico II

Alternative Name(s)
University of Naples Federico II, University of Naples, Federico II University of Naples, Università di Napoli, Università di Napoli Federico II, UNINA

Funding Body Type
Government organisation

Funding Body Subtype
Universities (academic only)

Location
Italy

Funder Name
Newcastle University

Alternative Name(s)

Funding Body Type
Private sector organisation

Funding Body Subtype
Universities (academic only)

Location
United Kingdom

Funder Name
University of Nottingham

Alternative Name(s)
The University of Nottingham

Funding Body Type
Private sector organisation

Funding Body Subtype
Universities (academic only)

Location
United Kingdom

Results and Publications

Individual participant data (IPD) sharing plan
Anonymised data from the study can be made available on request from Prof. Mario Siervo (mario.siervo@nottingham.ac.uk). The data is an Excel spreadsheet and is fully anonymised. The dataset is already available and will be made available for 3 years after the publication of the paper. To share the data the researchers will seek to have in place a material transfer agreement between institutions. The analyses will be decided on a case-by-case analysis of the proposal.

IPD sharing plan summary
Available on request

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article		10/02/2023	29/12/2023	Yes	No
Protocol file	version 2	20/01/2015	22/11/2022	No	No