

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

<b>Submission date</b> 07/10/2022	<b>Recruitment status</b> No longer recruiting	<input type="checkbox"/> Prospectively registered <input checked="" type="checkbox"/> Protocol
<b>Registration date</b> 28/12/2022	<b>Overall study status</b> Completed	<input type="checkbox"/> Statistical analysis plan <input checked="" type="checkbox"/> Results
<b>Last Edited</b> 29/12/2023	<b>Condition category</b> Other	<input type="checkbox"/> 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/m<sup>2</sup>), 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](mailto:mario.siervo@nottingham.ac.uk)

## Contact information

### Type(s)

Scientific

### Contact name

Prof Mario Siervo

### ORCID ID

<http://orcid.org/0000-0001-5515-0944>

### Contact details

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## **Additional identifiers**

### **EudraCT/CTIS number**

Nil known

### **IRAS number**

### **ClinicalTrials.gov number**

Nil known

### **Secondary identifying numbers**

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

### **Study design**

Two-arm open-label parallel randomized clinical trial

### **Primary study design**

Interventional

### **Secondary study design**

Randomised parallel trial

**Study setting(s)**

Other

**Study type(s)**

Prevention

**Participant information sheet**

Available on request from Prof. Mario Siervo ([mario.siervo@nottingham.ac.uk](mailto:mario.siervo@nottingham.ac.uk))

**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/m<sup>2</sup>) 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 measure

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

### Secondary outcome measures

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

### Overall study start date

05/12/2014

### 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/m<sup>2</sup>)
3. Non-smokers
4. Weight stable

### Participant type(s)

Patient

### Age group

Mixed

### Lower age limit

50 Years

### Upper age limit

75 Years

### Sex

Both

### Target number of participants

36

### Total final enrolment

**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**

England

Italy

United Kingdom

**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  
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## **Sponsor information**

**Organisation**

University of Naples Federico II

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**Sponsor type**

University/education

**Website**

<http://www.unina.it/index.jsp>

**ROR**

## **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)**

### **Funding Body Type**



Private sector organisation

### Funding Body Subtype

Universities (academic only)

### Location

United Kingdom

## Results and Publications

### Publication and dissemination plan

Results will be presented at scientific conference (UK Nutrition Society Annual Meeting). A paper will be prepared for submission in high-impact nutrition or metabolism peer-reviewer journal.

### Intention to publish date

30/11/2022

### 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?
<a href="#">Protocol file</a>	version 2	20/01/2015	22/11/2022	No	No
<a href="#">Results article</a>		10/02/2023	29/12/2023	Yes	No