ISRCTN36524222 https://doi.org/10.1186/ISRCTN36524222

Are inorganic nitrates protective for fatty liver?

Submission date
11/09/2017

Recruitment status
Stopped

Registration date
18/09/2017

Overall study status
Stopped

Last Edited
20/10/2021

Condition category
Nutritional, Metabolic, Endocrine

[ ] Prospectively registered
[ ] Protocol
[ ] Statistical analysis plan
[ ] Results
[ ] Individual participant data
[ ] Record updated in last year

Plain English summary of protocol

Background and study aims
The Metabolic Syndrome (MetS) is a cluster of cardiovascular risk factors (obesity, hypertension, insulin resistance, and dyslipidaemia) associated with inflammation, pro-thrombotic state and non-alcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH). Neither pharmacological (medications) approaches nor diet/exercise (due to low long-term compliance) have proven to be efficient in preventing/treating obesity and its comorbidities therefore new approaches are thus needed. Here we propose to nutritionally modulate nitric oxide (NO) signaling to revert the effects of the MetS. In rodents, nutritional supplementation with inorganic nitrate reduces weight and visceral fat, improves glucose metabolism, and promotes fat burning in organs such as skeletal muscle and adipose tissue. Nitrate-rich vegetables and extracts thus have potential as therapeutic interventions for MetS and NAFLD/NASH. In fact, NAFLD results from excessive storage of lipids, where the capability of the hepatocytes to store, burn and process the lipids is exceeded. Increasing fat burning in the liver has been proposed as a promising strategy for NAFLD management. Preliminary pre-clinical results show that inorganic nitrate promotes metabolic health and interferes with the mechanisms leading to NASH (i.e. fat accumulation, inflammation) through a mechanism of enhanced fat burning. This study aims to investigate whether a short term beetroot juice supplementation can improve the metabolic health of patients with fatty liver by lowering fat accumulation in the liver and interfering with the mechanisms that allow fatty liver progression and worsening.

Who can participate?
Men aged 30-55 years old who have a diagnosis of NAFLD/NASH.

What does the study involve?
Participants are randomly allocated to one of two groups. All participants are asked to revise their diet for the entire duration of the study. The first group consume a small bottle of beetroot juice twice a day. For the first 28 days this is a nitrate free drink and the next 28 days the juice will be nitrate-enriched. Those in the second group drink a small bottle of beetroot juice twice a day. The first 28 days the drink contains the nitrate and the next 28 days the drink is nitrate free. Participants are followed up four times to assess their body composition, lifestyle and liver function (through blood and urine tests).

What are the possible benefits and risks of participating?
There is no direct benefits to research participants. If the human nutritional trial confirms our

pre-clinical observations, we might observe an improvement in the metabolic profile of the markers of liver damage while the participants are on the nitrate-rich beetroot juice; on the other hand, we do not expect a net clinical therapeutic advantage (i.e. a complete regression of NAFLD) due to the short duration of the challenge. The main side effect of beetroot juice is beeturia (red urine) and red stool. This change in urine/faeces colour is not associated with any pathological state, neither is it a hallmark of disease.

Where is the study run from?
Addenbrooke's Hospital (Cambridge, UK)

When is the study starting and how long is it expected to run for?
April 2016 to September 2020

Who is funding the study?
Medical Research Council (UK)

Who is the main contact?
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Contact information

Type(s)
Scientific

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Additional identifiers

Protocol serial number
34696

Study information

Scientific Title
Beneficial effects of inorganic nitrates in liver steatosis

Study objectives
This study aims to investigate whether a short term beetroot juice supplementation can improve the metabolic health of patients with fatty liver by lowering fat accumulation in the liver and interfering with the mechanisms that allow fatty liver progression and worsening.

Ethics approval required
Old ethics approval format

Ethics approval(s)
East of England - Cambridge Central Research Ethics Committee, 08/05/2017, ref: 17/EE/0002

Study design
Randomised; Interventional; Design type: Dietary

Primary study design
Interventional

Study type(s)
Treatment

Health condition(s) or problem(s) studied
Diseases of liver

Interventions
The main intervention consists of drinking a small bottle of beetroot juice twice a day. The beetroot juice contains a high or low concentration of nitrates: neither the participant nor the research team will know in which order the participant is taking the two versions of the juice. Before starting the juice intervention and between the two treatments participants have a one-week washout period with no beetroot juice. During the study, the participants are also asked to revise their diet. Specifically, the nutritional intervention during this study (70 days) includes foods with low or absent nitrate levels.

Patients are randomised in two different arms: 15 patients are enrolled in the “Placebo-First” Arm (28 days of nitrate-free treatment followed by 28 days of nitrates-enriched treatment), the other 15 patients are enrolled in the “Nitrates-First” Arm (28 days of nitrate-enriched treatment followed by 28 days of nitrates-free treatment). The randomisation applied in this study is constrained and it is done through the website http://randomization.com/.

During the first visit consent form, clinical and lifestyle information as well as blood and urine samples are collected.
In addition to the first visit, 4 short visits are conducted to collect:
1. Anthropometrics
2. Lifestyle questionnaires
3. Body composition
4. Liver stiffness
5. Blood, and urine samples

Intervention Type

Other

Primary outcome(s)
1. The primary outcome will be assessed through the changes measured at baseline, 7, 35, 42 and 70 days and include:
1.1. Plasma 3-aminoisobutyric acid which will be measured using liquid chromatography-mass spectrometry (LC-MS),
1.2. Changes in gene expression and activity of the key metabolic transcription factors peroxisome-proliferator activated receptor 1 alpha and delta in peripheral blood mononuclear cells using reverse-transcription and quantitative polymerase chain reaction

Key secondary outcome(s))
1. The effect of nitrate supplementation on body composition measured using a bioelectrical impedance device at baseline, 7, 35, 42 and 70 days
2. The effect of nitrate supplementation on plasma lipidome using LC-MS at baseline, 7, 35, 42 and 70 days
3. The effect of nitrate supplementation on plasma and urine nitrate and polyphenols levels using mass spectrometry methods at baseline, 7, 35, 42 and 70 days

Completion date
01/09/2020

Reason abandoned (if study stopped)
Participant recruitment issue

Eligibility

Key inclusion criteria
Current participant inclusion criteria (as of 07/02/2018):
1. Men with clinical diagnosis of non-alcoholic steatohepatitis and a mild (F0-F2) fibrosis stage as predicted by a FIB-4 score < 2.67
2. Age between 30-55 years
3. BMI between 25-35
4. Triglycerides ≥ 1.7 mmol/L

Previous participant inclusion criteria
1. Men with clinical diagnosis of non-alcoholic steatohepatitis and a mild (F0-F2) fibrosis stage as predicted by a FIB-4 score < 2.67
2. Age between 30-55 years
3. BMI between 25-35
4. Glycaemia > 5.5 mmol/L
5. Triglycerides ≥ 1.7 mmol/L

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group

Adult

Lower age limit
18 years

Sex
Male

Key exclusion criteria
1. Current participation in other interventional clinical trials (to avoid confounding with other study outcomes), observational studies are allowed
2. Other hepatic diseases: i.e alcoholic (> 21U/wk), viral and autoimmune hepatitis (AIH, PSC, PBC), genetic conditions (e.g. hemochromatosis), drug-induced hepatitis, etc.
3. History of chronic conditions that could influence the study outcomes such as
3.1. Anaemia (Hb< 11g/dL) or abnormal values in WBC, platelet count, or INR
3.2. Glomerular filtration rate lower than 60 mL/min/1.73m2 or diuretics
3.3. Active cancer and any diagnosis of malignant cancer in the last 5 years
3.4. Chronic Inflammatory disease (e.g. IBD, rheumatoid arthritis) in chronic treatment with NSAIDs/Corticosteroids
3.5. Type 1 diabetes
4. Treatments that could influence the study outcomes such as:
4.1. Anti-Diabetic medications (Metformin, TZDs, insulin)
4.2. Lipid-lowering agents (Fibrates/Omega3)
4.3. Nitrate-derived agents
4.4. Anti-acids
4.5. Beta-blockers
4.6. Weight loss medications (sibutramine, orlistat, rimonabant) and history of bariatric surgery (weight loss related changes in systemic metabolism)
4.7. Hormonal therapies (oestrogens, thyroxine, and progesterone)
4.8. Anti-psychiatric drugs (antidepressants, sedatives, antipsychotics)
4.9. Sildenafil
4.10. Anticoagulants

Date of first enrolment
22/08/2017

Date of final enrolment
01/01/2020

Locations

Countries of recruitment
United Kingdom

England

Study participating centre
Addenbrooke's Hospital
Hills Road

Cambridge
United Kingdom
CB2 0QQ

Sponsor information

Organisation
Cambridge University Hospitals NHS Foundation Trust

ROR
https://ror.org/04v54gj93

Funder(s)

Funder type
Research council

Funder Name
Medical Research Council

Alternative Name(s)
Medical Research Council (United Kingdom), UK Medical Research Council, Medical Research Committee and Advisory Council, MRC

Funding Body Type
Government organisation

Funding Body Subtype
National government

Location
United Kingdom

Results and Publications

Individual participant data (IPD) sharing plan
Part of the datasets generated during and/or analysed during the current study will be stored in a publically available repository. Specifically, pseudonymised "Omics" data (including genomics and transcriptomics) will be available on dedicated platforms (e.g. National Center for Biotechnology Information, Gene Expression Omnibus, European Bioinformatics Institute, etc). These data will be available only if the patient will agree signing a consent form. Any residual biological material from this study will be kept in Prof Griffin’s Lab at the Department of

Biochemistry for a period of time of 15 years. These samples will be available to the scientific community (if requested) for further analyses (e.g. genes, metabolites) to generate more knowledge in the field of NASH and nitrates. The patients will provide signed permission for the use of the samples outside the remits of the actual project, upon agreeing to make these available, and the researchers will need to seek adequate ethical authorisation for further use without the need to ask consent of the patients for additional uses of their samples. Lastly, pseudonymised biological samples (urine and blood) will be analysed by Addenbrooke's Hospital (Cambridge, UK), University of Leeds (UK), University of Newcastle (UK), and University of Parma (Italy). For all the above mentioned points favourable ethical approval has been received.

IPD sharing plan summary

Stored in repository

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
HRA research summary			28/06/2023	No	No