PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	RANDOMISED, DOUBLE-BLIND, PLACEBO-CONTROLLED CLINICAL TRIAL INVESTIGATING THE EFFECTS OF INORGANIC NITRATE IN HYPERTENSION-INDUCED TARGET ORGAN DAMAGE: PROTOCOL OF THE NITRATE-TOD STUDY IN THE UK
AUTHORS	Lau, Clement; Hamers, Alexander Jozua Pedro; Rathod, Krishnaraj; Shabbir, Asad; Cooper, Jackie; Primus, Christopher; Davies, Ceri; Mathur, Anthony; Moon, James; Kapil, Vikas; Ahluwalia, Amrita

VERSION 1 - REVIEW

REVIEWER	Ignatios Ikonomidis National and Kapodistrian University of Athens,Medical School
REVIEW RETURNED	26-Oct-2019

GENERAL COMMENTS	In this interesting study protocol the authors aim to examine the effects of inorganic nitrate included in food on LV mass assessed by MRI and Pulse wave velocity in hypertensives using a double -blind, randomized, single -center, placebo -controlled phase II trial The study is well designed and the topic if of high interest and in accordance to the proposal for further research on the included in a recent consensus document of HFA ECVI and ESC WG of Aorta and Peripheral Circulation (Eur J Heart Fail. 2019 Apr;21(4):402-424. doi: 10.1002/ejhf.1436) suggesting that NO delivery may be a novel treatment target to improve both ventricular and arterial function improve ventricular- arterial coupling and thus increase myocardial work efficiency. As the authors suggest "…highlight the need for more studies that use other outcome measures (besides VO2) to evaluate the efficacy of nitric oxide donor agents in patients with HFpEF (e.g. cardiac function markers, biomarkers, and/or hospitalizations for HF) Thus the potential beneficial effect of dietary inorganic nitrate supplementation on ventricular- arterial coupling should be further discussed in introduction of this paper.

REVIEWER	Luke McIlvenna Victoria University Institute for Health and Sport Melbourne Australia
REVIEW RETURNED	29-Oct-2019

GENERAL COMMENTS	The Protocol from Lau et al, for the NITRATE-TOD study assessing long-term effects of inorganic nitrate supplementation on hypertensive target organ damage, outlines a comprehensive and well designed trial. This on-going trial has strong scientific underpinning and rationale building upon an extensive body of work carried out by the authors to date. minor comments: The addition measure of the nitrate and nitrite levels of the beetroot juice for each batch. Given that there can be variation in nitrate content between samples of the same product, this will allow quantification/validation the dose that is used (see Gallardo and Coggan, 2018 https://www.ncbi.nlm.nih.gov/pubmed/30299195) Given the long-term nature of the trial the storage of the beetroot juice needs to be taken into account with nitrate degradation occurring within 24hrs of storage at room temperature and after 4 days at 4°C. Levels remained stable for one month when stored at -20°C. (see Corleto et al, 2018 https://www.ncbi.nlm.nih.gov/pubmed/29660828). This may have already been taken in account. How is the adherence to the supplementation going to be assessed throughout the trial?

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Reviewer Name: Ignatios Ikonomidis

Institution and Country: National and Kapodistrian University of Athens,Medical School

“In this interesting study protocol the authors aim to examine the effects of inorganic nitrate included in food on LV mass assessed by MRI and Pulse wave velocity in hypertensives using a double -blind, randomized, single -center, placebo -controlled phase II trial

The study is well designed and the topic if of high interest and in accordance to the proposal for further research on the included in a recent consensus document of HFA ECVI and ESC WG of Aorta and Peripheral Circulation (Eur J Heart Fail. 2019 Apr;21(4):402-424. doi: 10.1002/ejhf.1436) suggesting that NO delivery may be a novel treatment target to improve both ventricular and arterial function improve ventricular- arterial coupling and thus increase myocardial work efficiency. As the authors suggest “…highlight the need for more studies that use other outcome measures (besides VO2) to evaluate the efficacy of nitric oxide donor agents in patients with HFpEF (e.g. cardiac function markers, biomarkers, and/or hospitalizations for HF)

Thus the potential beneficial effect of dietary inorganic nitrate supplementation on ventricular- arterial coupling should be further discussed in introduction of this paper.”

Thank you for highlighting this important issue. We have now added a sentence to the introduction and included the reference above (page 5 Line 12).

Reviewer: 2

Reviewer Name: Luke McIlvenna

Institution and Country: Victoria University

Institute for Health and Sport

Melbourne

Australia

“The Protocol from Lau et al, for the NITRATE-TOD study assessing long-term effects of inorganic nitrate supplementation on hypertensive target organ damage, outlines a comprehensive and well designed trial. This on-going trial has strong scientific underpinning and rationale building upon an extensive body of work carried out by the authors to date.

minor comments:

“The addition measure of the nitrate and nitrite levels of the beetroot juice for each batch.

Given that there can be variation in nitrate content between samples of the same product, this will allow quantification/validation the dose that is used (see Gallardo and Coggan, 2018 https://eur01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fpubmed%2F30299195&data=02%7C01%7C%7C6b4df9798c904863642208d75e1060cf%7C569df091b01340e386eebd9cb9e25814%7C0%7C0%7C637081296749167124&sdata=UFzeP%2FM48SI9x3nlHYPXxMczG0VC2aHEMBbdJwJjN8k%3D&reserved=0)“

Apologies we are measuring nitrate content in every batch given to patients, this detail has now been included in methods (Page 10 Line 27).

Given the long-term nature of the trial the storage of the beetroot juice needs to be taken into account with nitrate degradation occurring within 24hrs of storage at room temperature and after 4 days at 4°C. Levels remained stable for one month when stored at -20°C. (see Corleto et al, 2018 https://eur01.safelinks.protection.outlook.com/?url=https%3A%2F%2Fwww.ncbi.nlm.nih.gov%2Fpubmed%2F29660828&data=02%7C01%7C%7C6b4df9798c904863642208d75e1060cf%7C569df091b01340e386eebd9cb9e25814%7C0%7C0%7C637081296749167124&sdata=1aJ7Ua0dnzYCzGjK722owmhrCFSGmczh7VydvLfLV6U%3D&reserv ed=0). This may have already been taken in account.

We receive batches of matched juice from the supplier of nitrate containing and nitrate depleted juice. The nitrate content of the juice is normalised by the supplier. The concentration of each bottle of juice should be approximately the same. Measuring the nitrate content of each individual batch that is sent to each patient will allow us to verify this.

How is the adherence to the supplementation going to be assessed throughout the trial?

All patient volunteers are provided with a diary which needs to be completed and includes whether or not they took the daily juice. In addition, all patients return for a visit at 2 months for sample collection and general discussion about how the adherence is going. We have now included a statement in the methods stating this (Page 9 Line 22).

VERSION 2 – REVIEW

REVIEWER	Luke C McIlvenna Victoria University
REVIEW RETURNED	19-Nov-2019

GENERAL COMMENTS	All comments have been addressed Regarding the response to the following comment: Given the long-term nature of the trial the storage of the beetroot juice needs to be taken into account with nitrate degradation occurring within 24hrs of storage at room temperature and after 4 days at 4°C. Levels remained stable for one month when stored at -20°C. (see Corleto et al, 2018 https://pubmed.ncbi.nlm.nih.gov/29660828) The point being made was not about the concentration of the juice when it is received rather how it is stored e.g when the participant has it at home how it is stored and the length of time will influence the concentration/stability of the supplement. The study by Corleto shows that nitrate content is only stable for approximately 2 weeks at 4 degrees celcius then nitrate concentration drops and nitrite concentration increases. This is something to consider given the length of the supplementation period and the potential for this to influence the outcome of the study.

VERSION 2 – AUTHOR RESPONSE

Reviewer: 2

Reviewer Name: Luke C McIlvenna

Institution and Country: Victoria University

Please state any competing interests or state ‘None declared’: None declared

Please leave your comments for the authors below

All comments have been addressed

Regarding the response to the following comment:

Given the long-term nature of the trial the storage of the beetroot juice needs to be taken into account with nitrate degradation occurring within 24hrs of storage at room temperature and after

4 days at 4°C. Levels remained stable for one month when stored at -20°C. (see Corleto et al,

2018 https://pubmed.ncbi.nlm.nih.gov/29660828)

The point being made was not about the concentration of the juice when it is received rather how it is stored e.g when the participant has it at home how it is stored and the length of time will influence the concentration/stability of the supplement. The study by Corleto shows that nitrate content is only stable for approximately 2 weeks at 4 degrees celcius then nitrate concentration drops and nitrite concentration increases. This is something to consider given the length of the supplementation period and the potential for this to influence the outcome of the study.

In the article the reviewer is referring to it states that fresh vegetables were used for the juice that was not pasteurised and left in an open environment. Moreover, the authors state that it is bacteria that converted the nitrate to nitrite in these juice samples. The juice supplies that we use are pasteurised i.e. in a sealed bottle there is no bacteria. These beetroot juice shots have a shelf life at room temperature of approximately 8 months. The supplies sent to the volunteers provide sealed single doses in a bottle. Each bottle once opened will be consumed. As part of the trial volunteers bring at least one unopened bottle back and we then assess the nitrate content. In our experience there is no change in nitrate concentration in the supplies in all of the studies we have done previously that have lasted up to 6 weeks. The analysis of juice samples returned will clearly determine whether the juice supplies have been contaminated by bacteria. Our expectation is that this will not be the case. Patients are advised to store their juice in a cool place preferably in the fridge.