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The Effect of Chronic Nitrate Supplementation on Acute Mountain Sickness and Exercise Performance in Hypoxia

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Research Title: The effects of prolonged nitrate supplementation (beetroot juice) on Acute Mountain Sickness symptomology, and exercise performance in hypoxia

Research Co-ordinator(s): G. Rossetti, Dr S.J. Oliver & Dr J.H. Macdonald

Project summary and aims:
This study aims to investigate the effects of six days dietary nitrate supplementation (in the form of beetroot juice) on symptoms of acute mountain sickness (AMS) and exercise performance in simulated altitude. Recent studies have suggested that beetroot juice may have a beneficial effect on exercise performance in hypoxia (less oxygen in the air) by improving how the body delivers oxygen to where it's needed in the body and how efficiently it utilises that delivered oxygen. This has persuaded some professional sporting organisations to provide beetroot juice to their athletes, and has triggered some researchers to investigate whether giving beetroot juice can also decrease symptoms of AMS. However, some studies have shown no benefits of beetroot juice on performance, and theoretically beetroot juice supplementation may make AMS symptoms worse (not better). Determining whether beetroot juice has a beneficial or detrimental effect on AMS and exercise performance is thus important for populations such as athletes, tourists and the military who may consider its use.

Invitation to participate:
You have been invited to participate in this study. Before you agree to take part, it is important you understand the specific nature of the research and what will be required of you in this study. Please take your time and read the following information carefully. If you are unsure of anything please ask. Please take your time to decide if you wish to participate or not. You are welcome to discuss your choice with your friends, family or GP.

Am I eligible to take part?
Participation in this study is open to healthy men and women (18+ years) of a variety of ages and fitness levels. You will not be eligible to participate in this study if you have travelled to altitude (1500m+) in the last six months, or have any of the following conditions:

- Hypertension (systolic blood pressure >140 or diastolic >90 mm Hg)
- Cardiovascular disease
- Haematological disease
- Abnormally low haemoglobin concentration (males- below 13.5; females- below 12.0 g/dL)
- Chronic infectious disease
- Neurological or neuromuscular disease
- Musculoskeletal disorders or injury that are exacerbated by exercise
- Uncontrolled metabolic disease (e.g. diabetes)

- A history of kidney stones
- Respiratory problems (e.g. asthma, bronchitis)

Female participants must be post-menopausal, have a regular menstrual cycle, or be on the oral contraceptive pill for at least six months to be included in the study.

Do I have to take part?

The decision to participate in this study is entirely your own. If you choose to participate you will need to sign a participant consent form. After signing the consent form you are still free to withdraw from the study at any time. If you choose to not take part or take part but then withdraw from the study it will not affect your relationship with the School of Sport, Health and Exercise Sciences or any of the researchers involved in this study.

What will be required of you?

Participation in the study will require you to visit the Sports Science laboratory at Bangor University Normal site on eight occasions (total lab time of about 19 hours; see Table 1). The lab has an altitude chamber that looks like a conservatory built within a room. We will use the chamber to test your exercise performance at altitude, and to try to induce AMS. AMS is a self-limiting condition, which means it will resolve itself on its own with no long-term harmful effect on your health. AMS symptoms include headache, nausea, dizziness and fatigue. Symptoms you experience could range from mild to severe. If these symptoms become too burdensome, you are free to withdraw yourself from any test at any time.

During the testing phases you will be required to keep a food and drink diary. You will also be given a list of nitrate-rich foods such as leafy green vegetables that you should avoid during the study period. The researchers also ask that you refrain from using antiseptic mouthwash and other supplements whilst participating in this study. We will not prevent you from having caffeine if you normally do so, but please keep to your normal caffeine intake throughout the study. Additionally, please keep your caffeine intake the same across all of the days where you are required to come in for testing i.e. if you usually have one cup of coffee every morning, that's ok to continue, just make sure you have the same on each testing day and make a note of it in your food and drink diary.

Baseline tests

On days one and three of the study you will complete a maximal walking exercise test. On day one this will be in normal sea level conditions, and on day three this will be at a simulated altitude equal to 3225 m. For these tests you should wear shorts, t-shirt and trainers. When you arrive at the laboratory, you will first have your blood pressure measured; this will involve wearing an inflatable cuff around your arm. We will then take a pinprick blood sample from your earlobe, which we measure to determine how much haemoglobin there is in your blood (haemoglobin is the protein that transports oxygen). During testing, you will be taught to use a rating of perceived exertion scale (to tell us how hard the exercise feels to you). You will be fitted with a weighted rucksack (15kg for males, 12.5 kg for females), a heart rate monitor, a blood pressure cuff, a finger monitor that measures the oxygen concentration of your blood, and a special mask that does not restrict your breathing but measures the air you breathe out.

Once you are happy to begin, the treadmill will start at a brisk walking pace. As you walk the gradient will gradually increase (the speed will begin to gradually increase if you reach maximum gradient). You will be asked to continue until exhaustion; it is really important you keep pushing until you absolutely cannot continue. Once you have reached exhaustion, the test will stop. The test should last no longer than 25 minutes. The total time for these visits to the laboratory will be about 1.5 hours on day one, and 1 hour on day three.

Supplementation and testing phases

On day four you will have a venous blood sample taken by a trained phlebotomist. This will be used to measure the concentration of nitrate in your blood. You will then be given your first shot of beetroot juice or a placebo (a drink that looks and tastes the same but does not contain nitrate). You will be randomly assigned to receive either beetroot or placebo, and we will not tell you which you are receiving. You will be required to drink the shot once a day, at the same time of day for six days. This should be consumed in the morning 2.5 hours before any testing in the laboratory. One of the research team will call or text you each day when you are scheduled to take the supplement, so that you can make sure you take it at the correct time. After the six days, you will have a break of at least ten days that will act as a wash-out period before starting the second supplementation phase. The second supplementation phase will be the same as the first in every way (with the same tests), except your supplementation will be changed from beetroot to placebo, or vice versa. Again, we will not tell you which supplement you have been given until after the study has finished.

Moderate intensity exercise and altitude health tests (6 hours)

On day eight, after five days of supplementation you will have another venous blood sample taken. You will then complete a six-hour exposure to a simulated altitude equal to 4219m. You will need to bring a packed lunch with you. You must record this in your food and drink diary and make sure you bring exactly the same food both times. In this trial you should wear sports clothing and trainers but also bring some warm clothing with you (e.g. trousers, gloves and a warm coat). Before entering the chamber, you will be asked to insert a rectal thermometer (about the size of a phone charger cable) 6 cm past your rectum.

Whilst in the chamber, we will ask you to answer questions about how you're feeling to assess if you have any symptoms of AMS or more serious altitude illnesses. This will be at regular intervals when we will also check your blood pressure and oxygen concentration. During the simulated altitude exposure you will remain resting and will be able to read, complete written work or use a laptop computer.

In addition, we will ask you to complete three 20 min moderate intensity walking exercise bouts. You will complete these in the first, third and fifth hours of the altitude exposure. During the walking you will be loaded with the same rucksack (15kg for males, 12.5 kg for females) as the maximal exercise test and have the same measurements taken (how hard you feel the exercise is, heart rate, blood pressure, and analysis of the air you breathe out).

Exercise performance tests

On day nine (after six days of supplementation) you will have another venous blood sample taken. You will then complete another walking exercise task to exhaustion, at simulated altitude (3225 m, 14.1% O_2). For this trial you should wear the same clothes and trainers as the other exercise tests, and we will be taking the same measures from you as all the previous exercise tests. As before, please do your best to keep going until exhaustion. This visit to the laboratory will last about an hour.

After completing day nine (six days of supplementation) we will ask you to take a break lasting at least 10 days. After this break, you will begin the second phase of testing (approx. day 20 to 25) which will be a repeat of the tests you completed on days four to six, whilst you take a different supplement (beetroot or placebo, depending on which you had in the first supplementation phase).

Advantages of taking part

On completing the experiment you will be entered into a prize raffle, where you will have to opportunity to win either £50, £30, or £20 in Cotswolds vouchers!

Participation of the current study will also enable you to:

1. Exercise and experience simulated altitude. An experience that is both interesting and potentially beneficial to your training.
2. Get to know how you might cope with AMS at altitude.
3. Obtain key physiological knowledge of your body and training status.
4. Find out first hand if beetroot juice supplementation aids your performance.
5. Experience what it is like to participate in testing in a laboratory environment.

Disadvantages of taking part

There are some risks associated with participating in this study. These risks are:

Common (≥50%)

As this study aims to look at the effects of dietary nitrate on acute mountain sickness, the experimental protocol is intended to induce some symptoms of AMS. AMS is affected by individual susceptibility, so not everyone will experience these symptoms, but whilst in the chamber you are likely to experience some symptoms of AMS such as headache, nausea, dizziness and fatigue. This is normal and will go away once you leave the altitude chamber. Researchers will monitor you throughout and remove you from the chamber if your symptoms suggest you are developing a serious altitude illness. In addition, you are free to withdraw yourself from any test at any time.

Less common 1-5%

There is a chance (about 1 in 50) that venous blood sampling may leave a bruise on your arm. The risk of this will be minimised by using a trained phlebotomist.

Rare (less than 1%)

In any study there is a risk of a breach of confidentiality. To reduce this risk, all your data will be anonymised, all records will be kept in a secure storage area with limited access, and your information will not be released without your written permission.

Wearing a rucksack and completing exercise on a treadmill may lead to muscle strains or sprains. In order to minimise this risk, you will not be allowed to participant if you disclose any unresolved previous muscle injury. Trained researchers will be used and warm up and cool down exercise will be encouraged.

Rare but serious (less than 1%)

There is a chance (1 in 30,000 to 50,000) that venous blood sampling may cause infection or serious bleeding. The risk of this will be minimised by using a trained phlebotomist.

Maximal exercise testing is associated with a risk of sudden cardiac death. This risk is about 1 in 30,000. In order to minimise this risk, you will not be allowed to participant if you disclose any uncontrolled medical condition. Trained researchers will be used and warm up and cool down exercise will be encouraged. First aid equipment is also located in the laboratory and a first aid trained researcher will be present at all times.

Severe high-altitude illnesses: high altitude cerebral oedema (fluid on the brain); high altitude pulmonary oedema (fluid on the lung) and pericarditis (fluid on the heart) can occur when humans are exposed to low oxygen environments and are potentially fatal if left untreated. The exact risks of these conditions in this study are unknown but theoretically they could occur and their risk may be increased because of the maximal exercise and beetroot supplementation involved in this study.

Of 13 participants that have completed baseline tests and a full trial (9 having completed the study) so far, one participant one day after having completed the study reported chest pain and had to go to hospital. The symptoms may have been caused by fluid on the heart and may have been due to the present study. However the exact diagnosis and whether these symptoms were due to the study could not be confirmed.

In order to minimise the risk of severe altitude illnesses occurring to you, researchers will monitor you for signs and symptoms of these conditions throughout and after your time in the study and remove you if you show any sign of developing these illnesses.

Baseline tests		
Day 1 *~1.5 hours*	Day 2	Day 3 *~1 hour*
Consent Your characteristics Maximal exercise treadmill test at sea level	Rest day	Maximal exercise treadmill test at simulated altitude (*3225 m*)

Supplementation testing phase 1					
Day 4 ~15 mins	Day 5	Day 6	Day 7	Day 8 *7 hours*	Day 9 *~1 hour*
Blood sample (~1 tsp) 70 mL Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot Blood sample (~1 tsp) Moderate intensity treadmill exercise and altitude health tests *(4219 m)*	Beetroot juice or placebo shot Blood sample (~1 tsp) Treadmill exercise Performance test (*3225 m*)

Minimum 10 days break

Supplementation testing phase 2					
Day 20 ~15 mins	Day 21	Day 22	Day 23	Day 24 *7 hours*	Day 25 *~1 hour*
Blood sample (~1 tsp) Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot	Beetroot juice or placebo shot Blood sample (~1 tsp) Moderate intensity treadmill exercise and altitude health tests (*4219 m*)	Beetroot juice or placebo shot Blood sample (~1 tsp) Treadmill exercise performance test (*3225 m*)

Table 1. Schematic of day-to-day procedures involved in this study.

Who has reviewed this study?
This study has been reviewed by the SSHES Ethics Committee.

What if new information becomes available?

If new information becomes available that might influence your decision to be in the study you will be provided a new Participant Information Sheet and will be asked to sign a new consent form.

Confidentiality
All information collected about you in the course of this study will be kept strictly confidential. Any information that leaves the School will have your name and address removed so that you cannot be identified from it. It will not be possible to identify yourself in any report or publication of the study.

Feedback from you
If you have any difficulties or problems, please discuss these with the researchers in the first instance. SSHES is always keen to hear the views of research participants about their experience. If you would like to give independent feedback, please ask your researcher to provide you with Form 6 – Participant Feedback Form – from the Ethics Guidelines Handbook. Completion of this form is optional. The completed form should be returned to Prof Andrew Lemmey, Chair, SSHES Ethics Committee, SSHES, Bangor University, Bangor LL57 2PZ. All information will be treated in a strictly confidential manner.

What happens after I go home?

Typically symptoms of AMS will resolve within two hours. You may feel tired for approximately 24 hours. You may use pain relieving and non-steroidal anti-inflammatory drugs (e.g. paracetamol and ibuprofen). If you wish to do so please ensure you inform the researchers of this and seek medical advice (e.g. from a pharmacist) if you require additional information regarding these drugs. Note we cannot provide the drugs for you.

The researchers will contact you at certain points to ensure you are recovering well from the study. It is very important that you respond to this contact otherwise they will become concerned about you.

In addition, if you have any unexpected symptoms within 7 days of any laboratory test, you should seek professional medical help AND contact the researchers below (regardless of whether you think your symptoms are due to the study or not).

When the study is over, we will send you a summary of the results (if you wish) and we will be happy to discuss these further. Your personal identifiable information will be destroyed within 6 months. Your collated research data and samples will be given a code and stored for 10 years to be used by altitude researchers either to confirm our findings or to answer related

scientific questions. Any study interventions will not be provided to participants routinely at the end of the study.

What if I change my mind about taking part?

If for any reason you wish to withdraw from this study, you are free to do so at any point (even during a laboratory test) and no pressure will be put on you to remain in the study. If for any reason you lose the ability to consent to the research during the programme, then you would be withdrawn from the study. Identifiable data or blood samples already collected with consent would be retained and used for scientific study. No further data or blood would be collected. No other research procedures would be carried out on or in relation to you the participant.

Questions?

Please ask us if you have any questions. You should not sign the form consenting to take part in the study if you still have unanswered questions or any doubts. Please feel free to contact any of the researchers (contact details below).

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Sam Oliver
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01248 383965

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Thank you for taking the time to read this information sheet

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

1	Title of project	The effects of prolonged nitrate supplementation (beetroot juice) on Acute Mountain Sickness symptomology, and exercise performance in hypoxia
2	Name and e-mail address(es) of all researcher(s)	Samuel Oliver: s.j.oliver@bangor.ac.uk Jamie Macdonald: j.h.macdonald@bangor.ac.uk Gabriella Rossetti peuc11@bangor.ac.uk Samuel Little peu212@bangor.ac.uk

Please tick boxes

1 I confirm that I have read and understand the Information Sheet dated 25th July 2016 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2 (i) Students:
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. If I do decide to withdraw I understand that it will have no influence on the marks I receive, the outcome of my period of study, or my standing with my supervisor or with other staff members of the School. ☐

(ii) General members of the public:
I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason. ☐

3 I understand that I may register any complaint I might have about this experiment with Professor Tim Woodman, Head of School of Sport, Health and Exercise Sciences, and that I will be offered the opportunity of providing feedback on the experiment using the standard report forms. ☐

4 I agree to take part in the above study. ☐

Name of Participant ……

Signature …… Date ……

Name of Person taking consent……

Signature …… Date ……

WHEN COMPLETED – ONE COPY TO PARTICIPANT, ONE COPY TO RESEARCHER FILE