LEEDS METROPOLITAN UNIVERSITY

STAGE 2 - RESEARCH ETHICS APPROVAL FORM

All research carried out by students and staff at Leeds Metropolitan University must receive ethical approval before any data collection commences.

Notes

- All applicants MUST complete the Risk Checklist and Stage 1 - Research Ethics Approval Form prior to completing this Stage 2 - Research Ethics Approval Form. Following completion of the Risk Checklist and Stage 1 - Research Ethics Approval Form, if your research study was provisionally classified as Risk Category 2 or 3, you need to complete this form.
- Full details of the project are to be provided in this Stage 2. Where a question in the Risk Checklist was answered YES, please ensure that specific details are included in the appropriate box below.
- If a question does not apply to your project, insert 'Not applicable' or N/A.
- Help is provided for each question. Further help can be found in the Research Ethics Procedures document.
- You navigate through the form by using the tab keys. If you prefer to complete a normal Word document, you can unlock the form by selecting the 'Restrict Editing' button on the Developer tab, then click on 'Stop Protection'. The boxes should expand to allow space for your text.
- Spellchecking is not available in Word forms, so you may find it helpful to prepare your responses in a Word document and then copy these to this form.
- Ensure the form is completed in sufficient detail to allow the reviewer/s to judge the ethical issues raised by the study. Remember that the reviewer/s will be considering the following questions when reviewing your application in order to be able to give ethical approval:
  - is it ethical to conduct the research project and is the proposed method of investigation appropriate, thorough and ethical?
  - does the research project meet the requirements of the relevant Research Ethics Principles (Research Ethics Policy A2.4)?

TO BE COMPLETED FOR PROJECTS IN RISK CATEGORY 2 AND 3	
Your name	Oliver Shannon
Project title	The effects of beetroot juice on exercise performance, cognitive responses, and physiological functioning at simulated moderate and high altitude

1	**Project Overview**

Please give a brief overview of your study, including a summary of your aims and objectives.
Help: Describe the purpose of the research and what question(s) the project should answer.

Introduction:
Exposure to a hypoxic (i.e. low oxygen) environment, such as experienced on ascent to terrestrial altitude, has a profound deleterious effect on skeletal muscle energy metabolism (Vanhatalo et al., 2011), cognitive functioning (Ando et al., 2013), and exercise tolerance / performance (Masschelein et al., 2012). Similarly, the hypoxaemia and tissue hypoxia experienced during exposure to simulated or terrestrial altitude mimics the hypoxia afflicting patients with acute and chronic illness that compromise oxygen availability/ delivery (e.g. COPD, peripheral arterial disease, vascular dementia) (Kenjale et al., 2011).

Recent evidence indicates that the multi-functional signalling molecule nitric oxide (NO) plays an integral role in the response to acute hypoxic exposure and during high-altitude acclimatisation, with elevated concentrations of NO metabolites reported in Tibetan highlanders and acclimatised lowlanders (Levett et al., 2011). Conversely, lowlanders exposed to hypoxia initially exhibit reduced concentrations of exhaled NO (indicative of NO bioavailability), with the magnitude of this decline associated with susceptibility to

altitude illness (Droma et al., 2002).

Dietary nitrate supplementation has received great interest due its reported ability to increase NO bioavailability and offset some of the decline in physiological functioning experienced in hypoxia (e.g. Vanhatalo et al., 2011; Masschelein et al., 2012) or in clinical patients affected by conditions with a hypoxic component (Kenjale et al., 2011). Vanhatalo and colleagues (2011) reported reduced muscle metabolic perturbations as assessed via calibrated 31 P-MRS during leg-extension exercise in moderate hypoxia (14.5 % FiO2) following acute supplementation with nitrate-rich beetroot juice (9.3 mmol) versus a nitrate devoid placebo. Exercise tolerance (i.e. time to fatigue) was also restored to normoxic levels. Other authors have reported elevated muscle and arterial oxygenation (Masschelein et al., 2012), and improved time-trial performance (Muggeridge et al., 2012) in hypoxia. Current evidence indicates that nitrate supplementation may alter physiological functioning and exercise performance to a greater degree in a hypoxic environment relative to normoxia (Kelly et al., 2014). However, investigations comparing the physiological response to nitrate supplementation across different hypoxic 'doses' are lacking, and therefore it is unclear whether the degree of hypoxia influences the ergogenic effect of nitrate supplementation.

Interestingly, recent studies have reported increased pre-frontal cortex perfusion (Presley et al., 2011), and enhanced neurovascular coupling in response to visual stimuli (Aamand et al., 2013) subsequent to nitrate supplementation. Further, Thompson et al. (2015) reported an improvement in cognitive performance in normoxia, in particular the speed of decision making (i.e. response time), during a high-intensity intermittent exercise protocol at sea-level following nitrate ingestion. It was suggested that nitrate supplementation facilitated a better matching between cerebral oxygen demands and oxygen delivery, enhancing cerebral oxygenation and cognitive performance.

Hypoxia is known to elicit severe detrimental effects on various aspects of cognitive functioning, predominantly due to the reduced arterial oxygen saturation and hence oxygen availability to the brain (Ando et al., 2013). Given nitrate supplementation has been demonstrated to both a) increase arterial oxygen saturation in hypoxia (Masschelein et al., 2012), and b) improve cerebral perfusion/ cognitive functioning at sea-level (Presley et al., 2011; Aamand et al., 2013; Thompson et al., 2015), it is tempting to speculate that nitrate supplementation may help offset the decline in cognitive functioning experienced in hypoxia.

To date, only one study has investigated the effects of nitrate supplementation on cognitive functioning in hypoxia. Lefferts et al. (2015) found no effect of nitrate-rich beetroot juice on cognitive functioning nor global cerebral blood flow (middle cerebral artery blood flow) at rest in hypoxia (11.6 % FiO2). However, this study was limited in several respects, which make it difficult to draw firm conclusions. Firstly, a relatively modest nitrate dose was administered (~6.5 mmol), and plasma nitrate and nitrite were not measured, making it difficult to determine whether supplementation had the desired effect on NO bioavailabilty. Perhaps importantly, Lefferts et al. (2015) found no effect of nitrate supplementation on arterial oxygen saturation nor resting blood pressure, which differs from most previous investigations in this area when higher doses of nitrate have been administered or consistent elevations in plasma nitrite reported (Masschelein et al., 2012; Siervo et al., 2013). A greater nitrate dose may therefore be necessary to consistently alter physiological functioning and cognitive performance in hypoxia. Secondly, the study of Lefferts et al. (2015) only included rest in hypoxia. It is likely that nitrate supplementation may elicit different effects on physiological and cognitive functioning when an exercise element is also included, which remains unexplored. The nitrate-nitrite-NO pathway is particularly important for maintaining NO bioavailability when oxygen tensions and tissue pH are low, and activity of the oxygen-dependent L-arginine NOS pathway is suppressed (Kelly et al., 2014), such as extant during exercise in hypoxia. It is therefore possible that nitrate supplementation may facilitate better maintenance of NO linked cerebral processes including vasodilation, neurotransmission, and neurovascular coupling (Thompson et al., 2015) when an exercise component is also included prior to cognitive testing in hypoxia.

Aims:
1. To explore the effects of high dose (~ 15.2 mmol) dietary nitrate supplementation on physiological and cognitive functioning, and exercise performance at simulated moderate (~ 14.5 % FiO2, 3000 m) and high (~ 11.7 % FiO2, 4300 m) altitude.

2. Elucidate the effect of nitrate supplementation on subjective measures, including mental and physical fatigue.

2	Methodology

Please give a description of your methodology, including any data collection and analysis methods.
Help: Give an outline of your study here. If the project is complex, you can also submit your research proposal/protocol (no more than 2-3 A4 sides) if this would help the reviewer's understanding of the project. Include details of your (or your Research Supervisor's) appropriate skills and qualifications to carry out this research.

Participants

Nine healthy men who have not visited an altitude above 1500 m in the past 3 months will be recruited to take part in this study. Participants will be fully informed of the experimental procedures, and any associated risks. Full written informed consent will be obtained prior to testing (Appendix A), and a pre-screening will be completed to ensure suitability to participate, including a questionnaire (Appendix B). Only individuals classified as 'low risk' as per the 2014 American College of Sports Medicine (ACSM) guidelines for exercise participation will be allowed to participate. Participants will be asked to continue with their usual physical activity routine throughout the testing period. However, they will be asked to abstain from intense exercise for 24 hours before the experimental session. Participants will complete a food and exercise diary for 24 hours before the first experimental trial (Appendix C), during which time alcohol and caffeine consumption will be prohibited. The food diary will be used to replicate diet prior to each subsequent testing session. The consumption of nitrate-rich foods will not be restricted during the course of the study. However, participants will be asked to avoid use of antibacterial mouthwash and chewing gum throughout the testing period, as this has been shown to attenuate the reduction of nitrate in the oral cavity by commensal bacteria (Govoni et al., 2008).

Overview

Participants will visit the laboratory on six occasions within an 8 week period. On the first visit to the laboratory, participants will complete a pre-screening routine (see below) and complete an incremental running test to volitional exhaustion in normoxia to determine $\dot{V}O_{2max}$. The values obtained will be used to define training status, and elucidate suitable sub-maximal exercise intensities for subsequent testing sessions. Consistent with previous investigations in this area (e.g. Fulco et al., 2011; Masschelein et al., 2012; Kelly et al., 2014) a single normoxic $\dot{V}O_{2max}$ test will be applied to quantify fitness and sub-maximal exercise intensities, instead of assessments of $\dot{V}O_{2max}$ at the exercise altitudes. The former approach was deemed preferable to ensure a consistent walking speed across trials (and therefore similar muscle recruitment), minimise participant visits to the laboratory, and provide a more easily interpreted measure of aerobic fitness.

On the second visit, participants will complete familiarisation of the exercise protocol in normoxia, but without collection of physiological measures. This session is designed to accustom the participants with all experimental procedures.

Each subsequent session will involve exercise in a normobaric hypoxic facility (maintained sea-level barometric pressure, reduced FiO_2) including two visits at a simulated altitude of 3000 m (F_iO_2 ~14.5 %) and two visits at a simulated altitude of 4300 m (F_iO_2 ~ 11.7 %). These altitudes were selected to replicate the demands of exercise at moderate and high altitude equivalent to that experienced during trekking, mountaineering, and some high-altitude competitions (e.g. mountain stages in cycle events (~2500 m), Everest Marathon (~5000 m), Pikes Peak Marathon (~4300 m)). The third, fourth, fifth, and sixth visits will constitute the experimental trials, and will involve 45 minutes steady-state exercise at 45 % $\dot{V}O_{2max}$ followed by 3 km as fast as possible (i.e. a treadmill time-trial (TT)). All exercise will include uphill (10 % gradient) walking on a treadmill whilst carrying a 10 kg backpack. A similar protocol is presently being used at Leeds Beckett University to replicate the demands of mountaineering and high-altitude trekking,

and has also been used in other published literature (e.g. Fulco et al., 2011).

140 ml concentrated nitrate-rich (~ 15.2 mmol nitrate), or nitrate-deplete beetroot juice (~ 0 mmol nitrate) (BEET IT, James White Drinks Ltd., Ipswich, UK) will be ingested 3 hours 15 minutes before exercise on the day of testing. On trial at each altitude will be preceded by nitrate-rich beetroot juice, and one trial at each altitude will be preceded by nitrate-deplete beetroot juice (i.e. 2 x nitrate and 2 x placebo trials in total). We have previously administered an identical supplementation regimen prior to both hypoxic and normoxic exercise testing sessions, with no adverse side effects. Water will be provided *ad libitum* throughout the testing period.

Design

The study will utilise a repeated measures design, and trials will be performed in a randomised order to diminish any order effects. Each trial will be separated by a 5 - 10 days washout period, and will be performed at the same time of day to minimise the influence of circadian variance. Supplements will matched for taste and appearance and given double-blind (i.e. without the participant or main experimenter knowing the constituents).

Preliminary testing

Pre-screening

All participants will complete pre-screening in line with the 2014 ACSM Guidelines. This will initially involve a pre-screening questionnaire and resting heart rate and blood pressure readings (Appendix B). A blood test for resting glucose and cholesterol concentrations may also be taken. Participants will be classified as 'low' risk (able to take part in maximal exercise safely) if one risk factor is identified but the participant is otherwise free from other signs or symptoms of disease and has no history of disease. A small blood sample will also be obtained via venepuncture of an antecubital vein, for assessment of sickle cell trait (see section 6 – risks and benefits, for further details).

Incremental exercise protocol

Participants will be asked to complete an incremental treadmill running test in normoxia (at sea level). The test will provide useful information regarding participant fitness, and to determine relative exercise intensities for the main experimental trials via regression analysis of the VO_2-speed relationship.

Each treadmill test will be conducted in two parts. During the first part of the test participants will be asked to begin walking at an easy pace on a treadmill set to 10 % gradient, whilst carrying a 10 kg backpack The starting speed will be estimated based on participant training status. The speed will then be increased by 1 $km \cdot h^{-1}$ every three minutes. After each three minute stage participants will be asked to briefly mount the side of the treadmill, and a finger-tip blood sample will be collected to determine blood lactate concentrations. Throughout testing, samples of expired gas will be collected, and heart rate will be monitored via a chest worn heart rate monitor strap. This first phase of the test will be terminated before participants reach volitional exhaustion, and will provide information regarding heart rate, blood lactate production, and oxygen consumption (VO_2) at a range of sub-maximal exercise intensities.

Participants will then be given 10 – 15 minutes to recover before the second part of the test begins. The second part of the test is designed to determine $\dot{V}O_{2max}$, which is a measure of the ability to take oxygen from the atmosphere and then use it in the muscles to produce energy for exercise. During the second part of the test, the treadmill speed will be fixed at a level estimated based on participant training status. The participant will not wear a backpack during this stage. This is to allow running speeds to be obtained and ensure fatigue occurs in line with $\dot{V}O_{2max}$, and not due to muscle fatigue. The treadmill gradient will commence at 1 %, and then be increased by 1 % every minute. Participants will be asked provide a maximal effort and run for as long as they can. This is usually between 5 and 10 minutes. This test is demanding but, by definition, tolerable as participants are able to stop exercising as soon as it becomes too difficult. The second part of the test is continuous phase (i.e. no stop periods between stages of the test).

Familiarisation trial

Participants will be invited to complete a familiarisation trial on their second visit to the laboratory. This will involve replicating the experimental procedures outlined below, including familiarisation with the cognitive testing procedures. Physiological variables will not be monitored during the familiarisation trial. A familiarisation trial will ensure the participant if fully aware of what is required during testing, make certain they understand how to work the relevant equipment (e.g. adjusting treadmill speed), and determine whether sub-maximal exercise intensities are appropriate.

Experimental procedures

Protocol

A schematic representation of the experimental trials is presented in Appendix D. Participants will arrive at the laboratory ~ 1 hour 45 minutes after consumption of either nitrate-rich or nitrate-deplete beetroot juice. They will rest in the laboratory at sea-level (i.e. normoxia) for 30 minutes, after which they will enter the normobaric hypoxic chamber at a simulated altitude of either 3000 m or 4300 m. Participants will rest in hypoxia for a further 45 minutes. They will then conduct 45 minutes of steady-state exercise at 45 % of normoxic $\dot{V}O_{2max}$, followed immediately by 3 km as fast as possible (i.e. a treadmill time-trial (TT)). All exercise will include uphill (10 % gradient) exercise on a treadmill whilst carrying a 10 kg backpack.

Measurements

NO bioavailability

Three approaches will be applied for assessing NO bioavailability. Firstly, an 8 ml blood sample will be obtained from a vein in the antecubital fossa on arrival at the laboratory and at the end of the testing session. Blood will be collected into a tube containing lithium-heparin for subsequent analysis of plasma nitrate and nitrite (key NO metabolites) via ozone based chemiluminescence. At the same time point, a blood sample will also be obtained from the fingertip to allow comparison between capillary and venous nitrate and nitrite concentrations. Finally, at the same time points, a measure of exhaled NO will also be obtained to indicate pulmonary NO bioavailability (NObreath, Bedfont Ltd., UK).

Blood pressure and heart rate

Resting blood pressure on the upper arm will then be measured using an automated sphygmomanometer, and heart rate via a chest worn heart-rate monitor strap (Polar Electro Oy, Finland). Measures for blood pressure will be obtained on arrival at the laboratory. Heart rate will be monitored continuously throughout testing, with measurements taken during pre-hypoxic exposure rest, rest in hypoxia, during steady-state exercise, and immediately following the TT.

Arterial, muscle and cerebral oxygenation

A sensor will then be placed on the right gastrocnemius and forehead (pre-frontal cortex) for the monitoring of local tissue oxygenation and blood flow throughout the testing session by means of near infrared spectroscopy (NIRS) (Artinis, Netherlands). NIRS is a non-invasive method which measures the reflection of light at near-infrared wavelengths (680 – 800 nm) to continuously estimate local tissue oxygenation, as indicated by the relative fractions of oxygenated (O_2Hb) and deoxygenated (HHb) haemoglobin, and tissue blood flow (THb), which is a sum of O_2Hb + HHb (Neary, 2004). This technique will provide useful mechanistic data, regarding tissue oxygen consumption and blood flow between supplemented and non-supplemented exercise. NIRS data will be averaged over 5 minute periods for analysis at rest pre-hypoxic exposure, at rest in hypoxia, during exercise, and during all cognitive testing. Arterial oxygen saturation (Nellcor, Medtronic, USA) will also be assessed via finger-tip pulse oximetry during pre-hypoxic exposure rest, rest in hypoxia, during steady-state exercise, and immediately following TT.

Expired gas

Expired gas will be monitored throughout exercise via an online gas-analysis system (MedGraphics Ultima CPX, USA). Data will be averaged throughout the final 5 minutes of steady-state exercise and during the 3 km TT, and subsequently analysed for oxygen consumption (VO2), carbon dioxide production (VCO2), and substrate oxidation.

Cognitive testing
Cognitive testing will be conducted using an iPad-based cognitive testing software (Cambridge Cognition, Cambridge, UK) pre-hypoxic exposure, during rest in hypoxia, during steady-state exercise in hypoxia, and post-TT. Three tests will be used to assess pre-frontal cortex based functions at each time point.

Tests will include the Attention Switching Task, Rapid Visual Information Processing Task, and Spatial Span Task. Similar tests have been applied to assess the effects of beetroot juice and other nutritional interventions on cognitive functioning at sea-level, and have shown sufficient sensitivity to detect small improvements in cognitive functioning following nutritional intervention (Gilchrist et al., 2014; Thompson et al., 2015).

Attention Switching Task
Variations of the Attention Switching Task have been previously used to study the relationship between nutrient intake and information processing speed (Durlach et al. 2002; Irwin et al. 2013). Black arrows pointing either to the left or right of the screen will be presented, either on the left or right of a white background. At the top of the screen a categorisation header will be simultaneously presented which read either 'LOCATION' or 'DIRECTION'. In the case of the former, participants will be required to press a button on the left or right of the screen depending upon the location the arrow appeared. In the case of the latter, the choice of appropriate button will be based upon the direction the arrow is pointing. For example, if an arrow appears on the left side of the screen with the header of 'LOCATION', participants should press a designated button on the left hand side of the screen irrespective of the direction of the arrow. Conversely, an arrow pointing to the left appearing with the header 'DIRECTION' requires participants to press the left button irrespective of the location of the arrow. Tasks will be presented in a random order evenly distributed between congruent (the arrow direction and location matching) and incongruent (the arrow direction and location not matching) conditions, and require approximately 8 minutes to administer. Outcome measures will include response time and accuracy.

Rapid Visual Information Processing Task
The Rapid Visual Information Processing Task is a sensitive measure of sustained attention. A white box appears in the centre of the computer screen, inside which digits, from 2 to 9, appear in a pseudo-random order, at the rate of 100 digits per minute. Participants are requested to detect target sequences of digits (for example, 2-4-6, 3-5-7, 4-6-8) and to register responses using the press pad. Outcome measures for this test include response time and accuracy.

Spatial Span Task
Spatial Span assesses working memory capacity, and is a visuospatial analogue of the Digit Span test. White squares are shown, some of which briefly change colour in a variable sequence. The participant must then touch the boxes which changed colour in the same order that they were displayed by the computer or in the reverse order. The number of boxes increases from two at the start of the test to nine at the end, and the sequence and colour are varied through the test. Outcome measures for this test include response time and accuracy, and maximal span length recalled.

Scales
Subjective scales will be applied to assess perceived exertion, mental fatigue, mood, and symptoms indicative of acute mountain sickness (AMS).

Perceived exertion
Participants will be asked to rate their perceived exertion using a 15 point (6-20) RPE scale (Borg, 1982) (Appendix E) during steady-state exercise, and immediately following the TT.

Mental fatigue and mood
Scales will also be applied to assess mental fatigue and mood before the first set of cognitive tests, and immediately following all subsequent tests.

Firstly, participants will be asked “how mentally fatigued do you feel right now?” and “how energetic do you feel right now?” and asked to rate these by marking a vertical line on a 100 mm line on an A4 piece of paper with the end points labelled ‘not at all’ (left hand end) and ‘extremely’ (right hand end) (Appendix F). The value will be expressed as a percentage of the scale (100% = extremely).

Mood will then be assessed using the Brunel Mood Scale (BRUMS) (Appendix G), a questionnaire which is based on the Profile of Mood States (Terry et al., 2003). The questionnaire contains 24 items which are answered using a Likert scale (0–4; “not at all” to “extremely”). The items are divided into 6 categories (anger, confusion, depression, fatigue, tension, and vigour) and scored from 0 to 16 (raw score total). The scores for fatigue and vigour will be subsequently analysed.

AMS

The Lake Louise AMS Scoring system (Appendix H) will be used to provide an indication of any adverse reaction to hypoxia. Baseline symptoms will be recorded pre-hypoxic exposure, during rest in hypoxia, during the final 2 minutes of steady-state exercise, following TT exercise, and immediately prior to leaving the hypoxic chamber (i.e. following the final series of cognitive tests).

Skills and qualifications appropriate for carrying out the research

The main researcher on this project is a PhD student with experience performing exercise testing and research using human participants, including several previous investigations into the physiological and ergogenic effects of beetroot juice, both at altitude and sea-level. He will be assisted by students studying a degree within the School of Sport, who will also have experience of exercise testing and will undergo appropriate laboratory induction and health and safety procedures. The project will be overseen by Prof. John O'Hara, who has extensively tested and supervised practical laboratory work.

All researchers have experience performing exercise testing in the CRI Hypoxic Facility, including knowledge of relevant safety and emergency procedures. Likewise, all researchers will be familiar with and have experience using the key techniques used to monitor physiological variables during activity (e.g. online gas analysis system, pulse oximetry, heart rate monitor). NIRS will be used to provide an estimate of tissue oxygen consumption and blood flow. This involves the use of a small light emitting diode and sensor to estimate the proportion of oxygenated and deoxygenated haemoglobin and myoglobin in the muscle. This technique has no known risks, and has previously been used as part of our research in this area. The lead researcher has experience conducting computerised cognitive testing, and will also be guided by Dr. Emily Williams and Dr. Blossom Stephan, who have extensive research experience in this area.

Pre and post-exercise venous blood samples will be obtained from a vein in the arm. The lead researcher (Oliver Shannon), who has undertaken comprehensive phlebotomy training and is signed off as competent, will conduct all blood sampling. Finger-tip blood sampling will be used during preliminary testing. All researchers are trained in capillary blood sampling and the relevant best practice to ensure health and safety standards are maintained.

3	Ethical Considerations

Please give a description of the main ethical considerations involved in the study.
Help: All research projects will have ethical issues, and you will be asked later in the process on recruitment, voluntary participation and the right to withdraw, but highlight here the main ethical considerations for your study (which may concern, e.g., the type of participants, the sensitive nature of the study, the data collection process, a lone researcher carrying out research off-campus) and advise how you will address the main issues. If the project is funded, give details here, and whether there are any potential conflicts of interest involved in the study.

The main ethical consideration for testing is the potentially negative side effects associated with exercising in a hypoxic environment, including dizziness, fainting, nausea, vomiting, and headache (acute mountain sickness (AMS)). Participants will also experience a substantial reduction in exercise performance compared to sea level, due to the lower rates of oxidative energy production.

As the duration of hypoxic exposure is relatively short (< 2 hours) and the hypoxic dose is similar to that used in previous studies in our laboratory (simulated altitude of 3000 m and 4300 m), the occurrence of AMS is unlikely. However, specific precautions are in place in case of any adverse side effects. Arterial oxygen saturation and heart rate will be regularly monitored whilst participants are in the hypoxic facility. Furthermore, the Lake Louise AMS scoring system (Appendix H) will be used to monitor symptoms of AMS, and the participant may be evacuated from the facility and into normoxic conditions which will quickly eliminate the symptoms of AMS.

In case of emergency, there will be a first aid trained assistant in close proximity to the hypoxic facility at all times. Furthermore, the lead researcher has been trained in oxygen delivery by Prof. David Woods who is a qualified medic, in case of emergency.

Additional ethical considerations in this study include the use of phlebotomy for blood sampling, and the inclusion of brief periods of maximal and supra-maximal exercise. With this in mind, the exercise protocol and participant groups have been selected based around previous research, which has demonstrated the suitability of similar methods. A full outline of the risks and associated precautions taken are outlined in section 6.

4	Human Participants

If your study includes Human Participants (or their data), please give a description of who will be included.
Help: Please note this should include sample size/number of participants, whether the project will focus on any particular groups/individuals, if it will include any at risk or vulnerable participants, participants aged 16 years or under, etc. Please also specify your rationale for including / excluding groups of participants. If the research involves secondary data not in the public domain, give details in this section.

Nine males aged 18 - 45 who have not visited an altitude above 1500 m in the past 3 months will be recruited to take part in the proposed study (see section 2). The required sample size was determined via power analysis, conducted on the data of Lansley et al (2011), indicating that nine participants were required to provide an 80 % power to detect a ~ 2 % difference in time-trial performance at an alpha level of 0.05. Volunteers will be fit and healthy, and not currently taking (or have recently taken) any medications or supplements that may interfere with the study. All participants will be asked to complete a pre-screening questionnaire (Appendix B) prior to participation, and will be classified as 'low risk' according to the ACSM 2014 guidelines. Volunteers who are not deemed fit for this study (i.e. do not fulfil the 'low risk' ACSM criteria for exercise participation) will be excluded.

Summary inclusion criteria:
- Healthy males
- 'Low risk' according to ACSM criteria outlined in Appendix B

(includes age, weight, body mass index (BMI), history of smoking, physical activity, and blood pressure)
- Not currently taking or have recently taken prescription medication or a nutritional supplement
- Have not visited an altitude above 1500 m in past 3 months
- Absent of sickle-cell trait

5	Recruitment, Voluntary Participation, Consent and Right to Withdraw

If your study includes Human Participants, please give a brief description of the recruitment process, how you will ensure voluntary participation, if (and how) informed consent will be obtained prior to participants taking part in the study, and the right of withdrawal from the research process.

Help:

- This should include clear information on how participants will be identified, approached and recruited; whether the study will include any covert research or deliberate deception; whether help is required from a third party/ gatekeeper to access participants; what information you will give participants, etc.
- If expenses or any incentives are to be offered to participants, give full details.
- If your research involves students, colleagues and/or other employees then you must specify the rationale for this and how you will address issues of coercion or feelings of obligation.
- Regarding withdrawal from the study, discuss the different stages/dates a participant could withdraw or withdraw their data, and how they could do this.

Potential research participants will be recruited through use of posters (Appendix I), social media (e.g. Facebook, twitter), and via email contact with local and university sports clubs.

Participants will be free to withdraw from the study at any point, without fear of any negative consequences. All participants will be given contact details for the lead investigator, and may withdraw via email, telephone or personal contact. Participants will sign a form before participation, indicating that they are happy for any data collected to be used as part of the PhD thesis, Master's thesis, and any associated publications (Appendix B). Participants will be able to withdraw this consent at any point prior to publication, after which their anonymous data will be in the public domain.

The research will be open to any willing and physically suitable male volunteers, and thus there is a possibility that students or colleagues from within the university may volunteer to participate. As with all potential participants, any student or colleague who volunteers will not be pressured or coerced to participate, and it will be made clear that it acceptable to not participate or withdraw at any point if they wish. They will be informed of this right via the participant information sheet (Appending B)

This study will not involve covert research or deliberate deception. Participants will be informed about the supplements they will be taking during the trials via the participant information sheet. However, participants and the lead investigator will be unaware of the exact supplement provided on a given occasion (i.e. nitrate-rich versus nitrate-deplete beetroot juice will be given double-blind). This is designed to avoid any placebo effect or bias based around assumed differences in the treatment.

Participants will not be paid for their participation in the study. They will receive personalised feedback regarding their fitness tests and supplement responsiveness, if they wish.

6	Risks and Benefits

Please give a brief description of how, when and where the research will take place and whether there are any risks and/or benefits involved.

Help:

- This should include information on what participants will be required to do, the rationale for this and the level of risk involved.

- When considering risks, please refer to risks to the participants, the researcher, any other parties to the research; and also any health and safety issues for anyone involved (e.g., for lone researchers carrying out fieldwork)
- If participants will be exposed to ionising radiation, separate approval documentation must be submitted with this application.

Risk to the participants

The procedures and any associated risks will be explained to each participant prior to the study, and they will be given written information via a detailed participant information sheet (Appendix A). All participants will be given the opportunity to ask questions relating to the research before they provide written fully-informed consent. It will be made clear both verbally and in writing (Appendix B) that all volunteers have the right to withdraw at any time, without giving any reason, and the right to exclude their data prior to publication. After this point, anonymous data will be in the public domain.

All of the techniques which carry risk in this study have been previously employed in a safe and effective manner during the first three studies of my PhD. The risks associated and strategies in place to attenuate this risk are outlined below:

Phlebotomy

Venous blood sampling

Two small 8 ml blood samples will be drawn from a vein in the arm (pre-hypoxic exposure, and immediately following exercise), via venepuncture during each experimental trial. A single 4 ml blood sample will also be drawn during pre-screening to determine if sickle-cell trait is present. This technique is invasive, carries a risk of infection, and causes some mild discomfort to participants, with a risk of bruising afterwards.

The total volume of blood drawn is very small (16 ml per trial, 68 ml across trials) and is generally well tolerated by healthy individuals in such studies. A separate sample will be collected pre and post-exercise via different sterile needles, instead of using an indwelling cannula. The former is deemed preferable, to avoid any discomfort or risks associated with exercising with an indwelling cannula, and to allow the collection of a more externally valid performance measure.

The main researcher (Oliver Shannon) has undertaken full laboratory training in phlebotomy and signed off as competent by Mr. Ran Kurvits, who is the institutional phlebotomy trainer and assessor. All researchers will wear the appropriate protective equipment including laboratory coats and disposable gloves. The risk of infection will be minimised via the use of aseptic methods and sterile equipment. All material will be disposed of in a safe manner according to the laboratory procedures (e.g. segregation of sharps and softs into appropriate clinical waste systems). Blood samples will be immediately centrifuged, and plasma will be separated and stored in a secure freezer in the Carnegie Research Institute. This does not fall under the human tissue act, as plasma is regarded as acellular. Any samples not used in analysis or after analysis will be disposed of appropriately according to laboratory procedures.

Finger-tip (capillary) blood sampling

Capillary blood sampling will be used during preliminary testing to determine blood lactate concentrations, and during the experimental trials as a measure of capillary nitrate and nitrite concentrations. All researchers are trained in capillary blood sampling, and will use one-time safety lances and microvettes. An antiseptic wipe will be used to clean the area before testing, protective clothing will be worn throughout testing as above, and material will be disposed of in a safe manner according the laboratory procedures.

High-intensity exercise

During preliminary testing, participants will undertake one incremental tests to exhaustion on a treadmill. This is designed to elucidate maximal oxygen consumption ($\dot{V}O_{2max}$) and the physiological responses to various sub-maximal exercise intensities. During the experimental trials, participants will be required to perform a maximal effort (3 km as fast as possible) as a measure of exercise performance.

Maximal and supra-maximal exercise carries several risks. These include dizziness, fainting, nausea, vomiting, cardiovascular complications, and musculoskeletal injuries. There is also a small risk of infection via testing equipment, such as the face mask and via blood sampling (see 'phlebotomy'). Risk of infection is minimised by ensuring best practice at all times (e.g. sterile or new equipment).

Participants will undergo screening to ensure their suitability for participation, as outlined above, based on the 2014 ACSM Guidelines. Participants will be monitored continuously for signs of unusual distress, and are allowed to stop at any time they wish. The incremental exercise and the 3 km TT will feel very hard. However, participants should be accustomed to brief periods of intense activity, although the precise exercise environment is likely to be unfamiliar to them (i.e. hypoxia). Following exercise, participants should recover fully within 5 minutes. Also, by definition, the exercise intensities should be manageable, as participants may terminate (incremental exercise) or reduce the exercise speed (time-trial) if it feels too difficult. Similar exercise protocols are successfully being used within the department at present.

To avoid/ attenuate the risk associated with tripping and falling, participants will be attached to the stop cable of the treadmill, to immediately stop the treadmill if they move too far towards the back of the belt. A large crashmat will be placed behind the treadmill at all times during testing, as an extra safety precaution. Further, an experimenter will always be in close proximity to the participant to assist if they lose balance or fall.

Hypoxia

Participants will perform exercise in hypoxia (simulated altitude 3000 m and 4300 m), where oxygen availability is reduced from ~20.9 % (normoxia) to ~14.5 % (moderate simulated altitude) and ~11.7 % (high simulated altitude). The risks associated with exercising in a hypoxic environment include symptoms of AMS and a reduction in exercise performance. Further details on the risks of exercising in a hypoxic environment and the precautions in place to minimise any risk to participants is presented in Section 3 – Ethical Considerations.

Ingestion of dietary nitrate

There has been debate over the safety of consuming large quantities of dietary nitrate and nitrite, because of the potential formation of carcinogenic nitrosamines. However, studies investigating the health effects of moderate-dose nitrate supplementation have reported no effect on protein nitration. For example, Larsen et al. (2011) found no difference in tyrosine nitration following three days supplementation with 0.1 $mmol \cdot kg^{-1} \cdot d^{-1}$ sodium nitrate or a placebo. Likewise, Besco et al. (2012) observed no difference in the concentration of nitrated proteins in the urine of participants undergoing three days of supplementation with 0.16 $mmol \cdot kg^{-1} \cdot d^{-1}$ sodium nitrate or placebo.

Importantly, the potentially deleterious effects of nitrosation on human health are strongly inhibited by presence of antioxidants and polyphenols (Mirvish et al., 1998). Therefore, any risk associated with nitrate consumption is likely to be attenuated when supplementation occurs via the consumption of nitrate and antioxidant rich vegetables such as beetroot juice. In light of this evidence, the present expert consensus is that dietary nitrate supplementation via the consumption of nitrate-rich beetroot juice is highly unlikely to be damaging to human health (Jones et al., 2011; Lundberg et al., 2011). Indeed, some authors now believe that the health benefits associated with increased fruit and vegetable intake may be, at least in part, a product of consuming increased quantities of dietary nitrate (Jones et al., 2011).

It was deemed preferable to utilise a 'natural' supplement in the present study to avoid the potential risks which have been associated with nitrate salts, and to attenuate the formation of potentially harmful nitrogenous compounds. Acute supplementation with similar and greater amounts of nitrate-rich beetroot juice has been well tolerated by participants in other studies. A commonly reported side effect of consuming beetroot juice is a red/ pink discolouration of urine and faeces (beeturia). This is a harmless side effect of excreting unmetabolised betalain pigments. All participants will be made aware of this

possible side effect via the participant information sheet (Appendix A) so that they are not alarmed if it does occur.

Dehydration and over-heating
Participants will be unable to consume fluid during the steady-state exercise and TT efforts, given they will be wearing a facemask to facilitate gas analysis. This carries a small risk of dehydration and may increase the risk of over-heating, but is minimal given short TT duration (< 20 minutes). Exercise will be completed in a well ventilated laboratory with temperature controlled to 20 C and 50 % humidity. Furthermore, participants will be permitted water *ad libitum* until they commence exercise, and following exercise if desired. Diet and physical activity will be replicated prior to each experimental trial, to ensure experimental results are not confounded by nutritional or recovery status.

Risks to the investigator
Taking and analysing blood samples
Collecting and subsequently analysing blood samples creates a small risk of infection or contamination for the researchers.

All researchers involved in the collection of blood samples will be trained and signed off as competent by the institutional phlebotomy trainer. This will include knowledge of how to safely collect blood samples, whilst minimising any concomitant hygiene risk. For example, all blood handling will be performed whilst wearing protective clothing, such as laboratory coats and disposable gloves. Likewise, sampling will use sterile equipment, which will be disposed of once used via the approved institutional procedures. All stored blood samples will be safe and secure.

Hypoxia
The researchers will be required to spend time in the hypoxic chamber during experimental trials (~ 2 hours) (total duration ~ 8 hours per participant), at a simulated altitude of 3000 m (FiO_2 ~ 15 %) or 4300 m (FiO2 ~ 11.7 %).

As with participants, there is a small risk of the researcher responding negatively to the hypoxic stimulus. This may include dizziness, fainting, nausea, vomiting, and headache, potentially adding to form AMS. However, the chances of a negative response to hypoxia in the researchers are low, given brief duration of exposure (< 2 hours), and substantially lower oxidative energy demands relative to participants.

A minimum of two researchers will be present at all times during testing, and will monitor each other's condition. It is not necessary for both researchers to be present within the hypoxic chamber for the full duration of each trial. Therefore, one researcher at a time may periodically withdraw to normoxia, which will further reduce the risk of experiencing negative side effects to hypoxia and/or alleviate any symptoms of AMS.

Whilst in the hypoxic chamber, the arterial oxygen saturation of researchers will be monitored, and symptoms of AMS will measured via the Lake Louise AMS Score. If a researcher starts to feel unwell, they will immediately withdraw to normoxia. This will quickly alleviate any symptoms of AMS. If there are insufficient researchers to safely continue with the trial (< 2), the protocol will be stopped immediately. In case of emergency, there will be a first aid trained assistant in close proximity to the hypoxic facility at all times. The safety of both the participants and the researchers will be placed above the requirements of data collection at all times. Oxygen will also be on hand, in case of emergency. The lead researcher has been trained in oxygen delivery by visiting Prof. David Woods.

Laboratory equipment
The laboratory space within the hypoxic chamber is relatively small, and will contain several large pieces of equipment (e.g. treadmill, gas analysis equipment). There is therefore a possible risk of tripping and/or collision based injury whilst in the chamber.

Unneeded or unrelated items which present a potential hazard will be removed from the laboratory so as to minimise this risk. Equipment will be used in line with the manufacturer's instructions, and researchers will be signed off as competent by the learning support team prior to testing.

Benefits to the participant
Individuals may benefit from participating in the study if they are interested in learning more about whether beetroot juice may be a viable nutritional aid for improving their exercise performance at altitude. This may be particularly attractive for competitive athletes, or those interested in mountaineering and trekking.

As part of the testing, participants will also complete an incremental running protocol. Information will be obtained on participants VO_{2max}, and sub-maximal response to exercise. The participant will be provided with these data, and given personalised feedback, should they wish.

7	Personal Data, Anonymity and Confidentiality

Please specify what type of information/data will be collected/analysed and the source(s). In addition, specify if and how you will ensure the anonymity of participants and keep information confidential.
Help: This should include information on whether you are collecting new information/data or using that that is already in the public domain; whether the data you are using includes personal details; how the data will be processed and stored; who will have access to it; how and when it will be destroyed; the Data Protection requirements for any sensitive personal data, etc. In addition, include whether there may be any requirements for disclosure of information to other parties due to professional practice or legal reasons. If there are limits to confidentiality, explain clearly how the participants would be advised about these limits and possible outcomes.

The names of all participants will be kept confidential, and are only know to the principle investigator. Participants will be allocated a participant number for the duration of the study, and all stored samples (e.g. plasma) and data will be labelled with this code to maintain anonymity. The key matching participant name and number will be accessible only to the principle investigator, and will be stored in on a password protected computer in a locked room.

The data obtained will be classed as 'new', as it is not currently in the public domain. Permission will be obtained before participation to use the data as part of my thesis and any publications which may result from this (Appendix B). Permission will also be sought, to allow Masters students to use sub-sets of these data for their major independent studies. Data will generally be presented as mean group values. However, if it is seen as beneficial to refer to individual results (for example, to identify a non-responder vs. responder phenomenon) participants will be referred to only by numerical identification (e.g. Participant 1.). Data will not be presented at any point with real names. Data will be stored for 10 years after the end of the study, after which time it will be destroyed.

Results will not be shared by the investigator with other participants.

Confidentiality is regarded with utmost importance. However, if abnormal values are noted which may indicate an adverse health condition, participants will be informed and asked to visit their general practitioner. They will be informed of this in the participant information sheet (Appendix B).

8	Reporting and Dissemination

Please give details of the planned dissemination and specify if the findings from the research will be

published and whether any permission is required for this.
Help: This should include information on the methods of dissemination (e.g., dissertation/thesis) and/or what will be published and where. Specify if any permission is needed (e.g., from participants, clients, gatekeepers, etc.) prior to publication, and whether there are any potential issues relating to Intellectual Property Rights when creating or using materials.

This study will constitute part of my doctoral thesis, and is intended for publication in a relevant peer-reviewed journal and at national and international conferences. Permission will be obtained from participants prior to participation to use their anonymised data in this manner (see section 7). These data may also be used by Masters and/or undergraduate students for their Major Independent studies.

9	Projects taking place outside of the UK

Will part or all of the research take part outside of the UK?

YES ☐ **NO** ☒ **If yes, give details below.**

Help: If yes, please specify where the research will take place and what will be involved. Research must comply with the laws of the country where it is taking place and also comply with local Data Protection and Intellectual Property legislation: you must confirm that your research is compliant with local requirements and how you have ascertained this. Advise if the project requires ethical approval in-country and how this has been ascertained. If approval is required, a copy of this should be included in the application or details of the process of how it will be obtained. Please also make reference to insurance and indemnity cover for the project.

N/A

10	Collaborative Projects

Is the research is a collaborative project (i.e., it involves more than one institution)?

YES ☒ **NO** ☐ **If yes, give details below.**

Help: If yes, please specify the other institutions involved and if ethical approval needs to be / has been given by them. Please also specify what procedures have been put in place to ensure ethical compliance from all partners.

Plasma analysis will be performed in partnership with colleagues at Newcastle University (Dr. Mario Siervo). Advice on interpreting the results of cognitive testing will also be provided by Dr. Blossom Stephan, from Newcastle University. So as to maintain confidentiality, all plasma samples will be labelled with the participant number, and no other identifying feature. Participant's names and other confidential data will not be shared with external collaborators.

11	Any other permission or external ethical approval required to undertake the project

Please specify if the project requires any other ethical approval or permissions not mentioned previously in this application and how and when these will be obtained.
Help:

- Other permissions: ethical approval does not give the right of access to the University's students, staff or the use of University premises to carry out research, and you may need to contact an appropriate University gatekeeper for agreement to approach potential participants or for the use of premises, so please give details.
- Gatekeepers: permission of a gatekeeper for initial access to participants may be required or to carry out data collection on their premises.
- If your project requires approval from an external ethics committee, eg, the National Research Ethics Service, this should normally be obtained prior to submitting this application.
- If a Disclosure and Barring Service check is required due to the specific participant group, give details.
- Regarding insurance and indemnity cover, some projects will require individual confirmation of cover. See the Research Ethics Procedures document for more details.

N/A

FOR PROJECTS INVOLVING RISK CATEGORY 2 AND 3: DECLARATION AND SIGNATURE/S			
APPLICANT (STUDENT/STAFF MEMBER/RESEARCHER)			
I confirm that I will undertake this project as detailed in stage one and stage two of the application. I understand that I must abide by the terms of this approval and that I may not make any substantial amendments to the project without further approval. I understand that research with human participants or their data must not commence without ethical approval.			
I have read an appropriate professional or learned society code of ethical practice:		Yes ☐ N/A☐	
Where applicable, give the name of the professional or learned society:			
Signed		*Date*	

RESEARCH SUPERVISOR/DIRECTOR OF STUDIES RECOMMENDATION FOR STUDENT PROJECTS					
I confirm that I have read stage one and stage two of the application. The project is viable and the student has appropriate skills to undertake the project. Where applicable, the Participant Information Sheet and recruitment procedures for obtaining informed consent are appropriate and the ethical issues arising from the project have been addressed in the application. I understand that research with human participants must not commence without ethical approval. I recommend this project for approval.					
Name		*Signed*		*Date*	

Local Research Ethics Co-ordinators

*Please complete EITHER **A** (giving ethical approval for the project) OR **B** (recommending the project to the Faculty Research Ethics Committee for approval)*

A	**LOCAL RESEARCH ETHICS CO-ORDINATOR APPROVAL** *For projects approved by the Local Research Ethics Co-ordinator*				
I confirm ethical approval for this project					
LREC Name		*Signed*		*Date*	

OR

B	**LOCAL RESEARCH ETHICS CO-ORDINATOR'S RECOMMENDATION FOR FACULTY APPROVAL** *For projects that require Faculty level approval*				
I recommend this project for consideration at faculty level. It cannot be approved at local level due to the following reason(s)					
LREC Name		*Signed*		*Date*	

Faculty Research Ethics Committee

For projects approved by FREC please complete the box below.

PROJECTS APPROVED BY THE FACULTY RESEARCH ETHICS COMMITTEE					
I confirm that this project was considered by the Faculty Research Ethics Committee and has received ethical approval					
Chair		*Signed*		*Date*	

This form will be retained for the purposes of quality assurance of compliance and audit for THREE years

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SUPPORTING DOCUMENTATION: what to submit with the application

For projects involving human participants, you must submit, where appropriate, the Participant Information Sheet/s and consent form/s. You must also submit every communication a participant will see or receive. Failure to do so will cause delays to the application.

Below is a checklist reminder of what could be submitted, depending on the research project. Please tick the appropriate boxes for each attachment or give details of the document at the end of the checklist.

SUBMISSION CHECKLIST	**Tick box**
RISK CHECKLIST AND STAGE 1 – RESEARCH ETHICS APPROVAL FORM	☐
STAGE 2 – RESEARCH ETHICS APPROVAL FORM	☐
Participant Information Sheet(s)	☐
Consent Form(s)	☐
Assent Form (usually for children participants)	☐
Recruitment documents *eg, posters, flyers, advertisements, email invitations, letters, web pages if online research*	☐
Measures to be used *eg, questionnaires, surveys, interview schedules, psychological tests*	☐
Screening questionnaire	☐
Letters/communications to and from gatekeepers/third parties	☐
Evidence of any other approvals or permissions *eg, NHS research ethics approval, in-country approval for overseas projects*	☐
Research proposal/protocol (no more than 2-3 A4 pages) *It is not a requirement that this is included, however, if this would help the understanding of a complex project by the reviewer(s), please include*	☐
Risk assessment form *Some projects may require a risk assessment form: see the Procedures document for details (eg, projects involving a physical intervention, collecting data off-campus)*	☐
Approval documentation for projects involving ionising radiation	☐
Confirmation of insurance and indemnity cover *Some projects need to be referred to the Insurance & Risk Officer: see the Procedures document for details*	☐
Other: give details here:	☐
	☐

SUBMITTING YOUR FORMS

- Students: email the typed forms (stage one and stage two) and supporting documentation to your Research Supervisor or Director of Studies.
- Staff: email the typed forms (stage one and stage two) and supporting documentation to your Local Research Ethics Co-ordinator.

Participant information sheet

Study title: The effects of beetroot juice on exercise performance, cognitive responses, and physiological functioning at simulated moderate and high altitude

Location: CRI Building, Carnegie Faculty, Leeds Beckett University

Investigators: Oliver Shannon (PhD student)
Prof. John O'Hara (Professor)
Dr. Lauren Duckworth (Senior Lecturer)
Dr. Matthew Barlow (Senior Lecturer)

Invitation to participate
You are invited to take part in a research study being performed at Leeds Beckett University. Before you decide whether you would like to participate, it is important that you understand why the research is being performed and what it will involve. Please take time to read the following information carefully and discuss it with friends and/or relatives if you wish. Contact details for the lead investigators are available at the end of this information sheet. Please feel free to ask us if there is anything that is not clear, or if you would like additional information. Take as much time as you want to decide whether or not you wish to take part.

What is the purpose of the study?
A number of recent studies suggest that beetroot juice may be a promising nutritional aid for individuals exercising in hypoxia (i.e. simulated altitude), by reducing the oxygen cost of exercise, extending the time to fatigue, and enhancing time-trial performance. The precise mechanisms through which beetroot juice may elicit these effects is not fully understood, but is believed to be related to an increase in the availability of nitric oxide (NO), which is an important signalling molecule in the human body.

Previous studies have investigated the effect of beetroot juice on individuals performing cycling, running, or leg extension exercise in varying degrees of hypoxia (2500 – 5000 m simulated altitude). Indeed, we recently observed a 3.2 % (10.9 second) improvement in 1500 m running performance at a simulated altitude of 2500 m following consumption of beetroot juice. However, further investigations are required to understand the precise conditions for which beetroot juice may be beneficial, and the extent of the beneficial effects of beetroot juice.

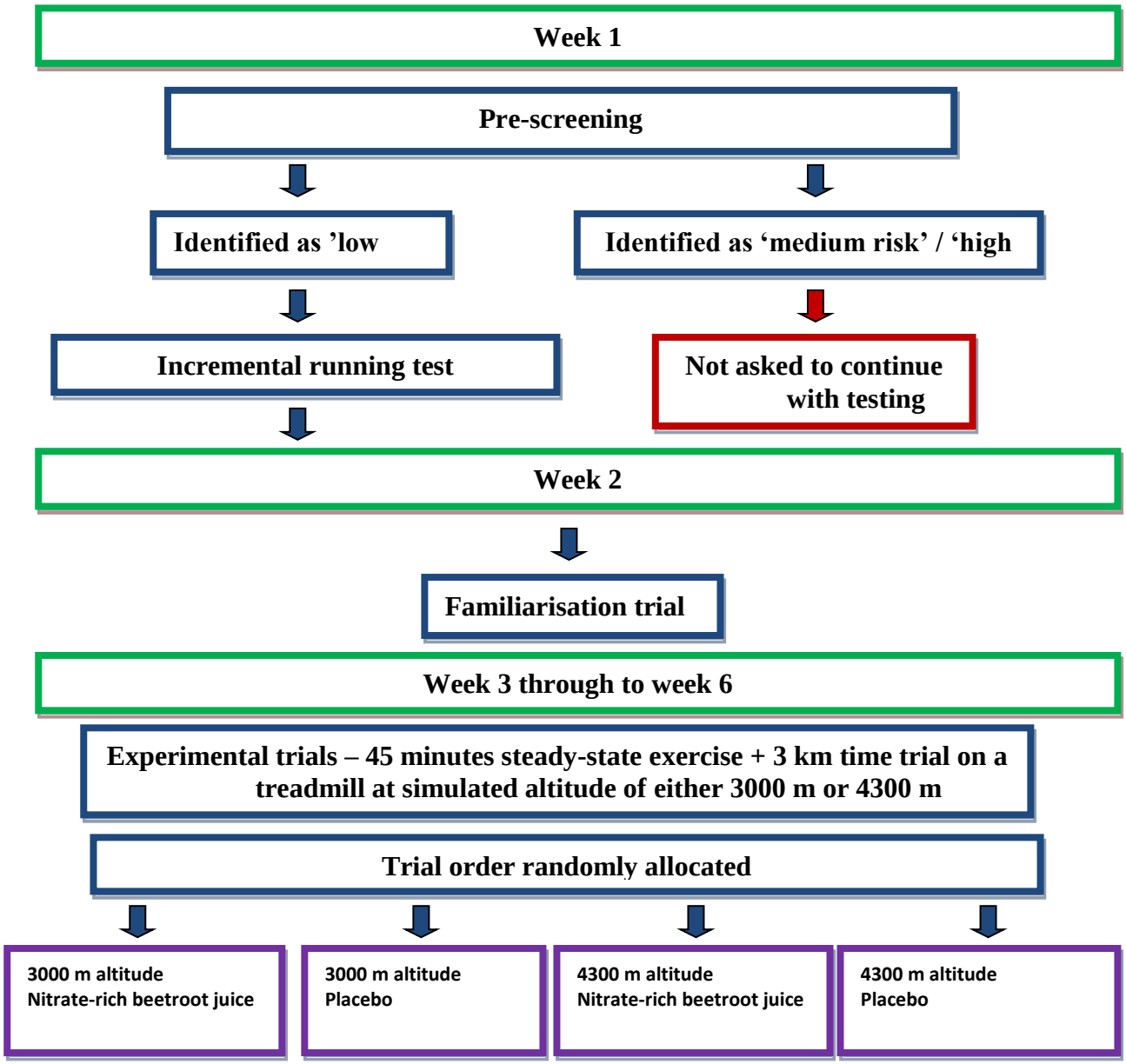
One key question which remains unanswered is whether the degree of hypoxia (i.e. the height of altitude simulated) influences the response to beetroot supplementation. Theoretically, beetroot juice may have a greater effect on physiological functioning and exercise performance at high versus moderate altitude, although this has not yet been directly studied. We are also interested in assessing the effects of beetroot juice on cognitive functioning (via a series of iPad based tasks), which is known to decline at altitude.

Therefore, this study aims to investigate the effects of drinking a concentrated beetroot 'shot' before exercise at moderate and high simulated altitude.

Why have I been chosen?
We are looking for nine health males between the ages of 18 and 45 to take part in this study. The inclusion criteria require that you are fit and healthy, and not currently taking any prescription medication or dietary supplements, as this may interfere with the study.

Do I have to take part?
No, it is your decision whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving reason and without any negative consequences.

What will happen if I take part?
Should you agree to take part in this study, you will be asked to visit the laboratory on six occasions. Testing will take place within the Environmental Chamber at Leeds Beckett University over an eight week period, and will involve the following:



Week 1: Pre-screening and preliminary testing

During your initial visit you will be asked to complete a pre-screening assessment to ensure you are low risk to perform exercise testing, according the ACSM (2014) guidelines. This will include completing a questionnaire, and resting blood pressure and heart rate measurements. To be defined as low risk, you must possess less than two risk factors for cardiovascular, metabolic and renal disease, and have no signs or symptoms or history of cardiovascular, metabolic and renal disease. Individuals identified as moderate or high risk will be excluded from the study, for safety reasons.

A small (4ml) blood sample will also be collected from a vein in the arm, and this will be analysed for sickle-cell trait. Sickle-cell trait is a blood condition known to compromise oxygen delivery. If you possess sickle-cell trait, exercising in simulated altitude may be unsafe, and you will therefore be excluded from the study for safety reasons. You will also be referred to your GP if a positive test result is established.

This will be followed by a treadmill fitness test to determine maximal oxygen uptake ($\dot{V}O_{2max}$), which is a measure of how much oxygen your body can take up and use during exercise. This test involves walking and running on a treadmill whilst the speed and/or gradient is gradually increased. You will be asked to exercise to volitional exhaustion, which means exercising until you feel you have reached your maximum and cannot go any further.

Throughout the test you will be asked to wear a facemask so that expired air can be collected and measured using an online gas analysis system. We will also monitor heart rate using a chest worn strap, and we will take five to eight fingertip blood samples (the exact number depending on how long you exercise for).

Week 2: Familiarisation

Approximately one week later, you will be asked to conduct a familiarisation trial. This will replicate the protocol of the main trials (see below), but will be conducted at sea-level and without any physiological measures taken. The aim of this trial is to help you become familiar with the testing procedures, and help us determine whether the exercise intensities are appropriate.

Week 3, 4, 5 and 6: Main trials

Your third, fourth, fifth, and sixth visits to the laboratory will serve as the main experimental trials, and will involve 45 minutes of steady exercise at a relatively light intensity (45 % $\dot{V}O_{2max}$), followed by 3 km as fast as possible (i.e. a time-trial). Exercise will be conducted on a treadmill set to a 10 % gradient, whilst carrying a 10 kg backpack. This is designed to replicate the demands of trekking at high altitude, and has been used in some of our previous studies within the university. Exercise will be performed in a special laboratory which replicates the environmental conditions of exercising at altitude. The oxygen concentration in the air will be reduced from around 20.9 % at sea-level to approximately 14.5 % (trials at 3000 m) and 11.7 % (trials at 4300 m).

On the day of testing you will be asked to consume 140 ml concentrated beetroot juice around 1 hour 45 minutes prior to arriving at the laboratory. After drinking the beetroot juice, you may consume water to thirst, but are asked to abstain from other types of drink (e.g. tea, coffee, squash)

When you arrive at the laboratory, we will collect pre-exercise measurements while you rest in a comfortable chair. These will include a small blood sample (8 ml) from a vein in your arm and a fingertip blood sample, heart rate, blood pressure, arterial oxygen saturation (via a probe on the finger), and exhaled NO measurements. We will also use the Lake Louise AMS Scoring system to monitor symptoms of acute mountain sickness at this point. Venous blood sampling will occur via venepuncture, which involves briefly inserting the tip of a needle into a vein in your arm. This is

virtually painless, and is generally well tolerated by healthy participants. If you have a needle phobia, please discuss this with the researchers before attending the laboratory.

Sensors will be placed on your calf muscle and forehead, to provide information about the supply of oxygen to your muscles and brain. We will also ask you to complete a series of iPad based metal tasks (cognitive functioning test) which will assess how quickly and accurately you perform the task. We will ask you to rate how mentally fatigued you are, and rate your mood using a questionnaire before and after the cognitive tests.

We will then enter the hypoxic chamber (where we will be at a simulated altitude of either 3000 m or 4300 m), and you will be asked to rest for 45 minutes. During this period, we will repeat the cognitive tests, ask you to rate your mental fatigue, mood, symptoms of acute mountain sickness, and measure your arterial oxygen saturation and heart rate.

Once the rest period is over, an exercise protocol will then commence. This will involve 45 minutes of steady-state moderate intensity walking at 45 % of your sea-level $\dot{V}O_{2max}$. We will ask you to repeat the cognitive tests during this period. Steady-state exercise will be followed by 3 km as fast as possible (i.e. a treadmill time-trial). During the treadmill time-trial, you will be able to control the treadmill speed, and will be given notice of how far you have covered. Treadmill running speed will not be visible, to avoid pacing. All exercise will involve walking up hill (10 % treadmill gradient) with a 10 kg backpack. Expired gas will be monitored throughout exercise by breathing through a sterile non-restrictive face mask. We will also monitor your arterial oxygen saturation and heart rate. You will also be asked to rate the difficulty of exercise using a standard Ratings of Perceived Exertion (RPE) Scale.

Following exercise, you will be asked to repeat the iPad based mental tasks (cognitive test), and rate your mental fatigue, mood, and symptoms of acute mountain sickness. You will then be asked to rest in the chamber for a further 10 minute whilst we obtain an additional venous and fingertip blood sample, and measure your exhaled NO. You will then be free to have a shower in our facilities, and leave at your leisure.

Pre-trial requirements

You may continue with your usual physical activity routine throughout the testing period, but will be asked to abstain from intense exercise for 24 hours before each main trial. This is to ensure you are not fatigued for testing. In the 24 hour period before each main trial, you will also be asked to complete a food and exercise diary. During this time period, you will be asked to avoid the consumption of alcohol and caffeine, and you will be asked to avoid antibacterial mouthwash and chewing gum, which have been shown to reduce the potency of the beetroot supplement. We will provide you with some beetroot juice to drink 1 hour 45 minutes before your visit.

What are the possible benefits of taking part in this study?

The study is being undertaken for research purposes rather than to attempt to find the causes of disease, or a cure for a disease. You may benefit from participating in the study if you are interested to learn more about your ability to optimise exercise performance at altitude and whether beetroot juice is worth using in such environments.

What are the possible risks and discomforts?

Exercising in hypoxia

All experimental trials will be conducted in the Environmental Chamber at Leeds Beckett University.

The environmental chamber allows us to control the amount of oxygen available in the air you breathe. During exercise, we will reduce the amount of oxygen available in the chamber to stimulate the effects of exercising at altitude (from 20.9 % at sea level to around 14.5 % at 3000 m and 11.7 % at 4300 m). This is known as normobaric hypoxia, and involves replacing some of the oxygen in the air with nitrogen. As there is less oxygen available, you will likely feel out of breath quicker than you usually do when exercising.

Exercising in this environment can be associated with feelings of dizziness, nausea, fatigue and weakness, and a headache, which may amount to acute mountain sickness (AMS). As the exposure to hypoxia is relatively brief (< 2 hours), severe symptoms of AMS are unlikely to develop. However, if you do feel unwell at any time, you can stop exercising and leave the chamber. This will quickly alleviate symptoms of AMS. We will carefully monitor how you feel at all times, and will measure the amount of oxygen circulating in your blood (arterial oxygen saturation), using a sensor placed on your finger tip to make sure you are safe. You may choose to stop exercise and leave the chamber at any point you wish. We may also stop exercise testing immediately if the researchers feel you should not continue.

Preliminary testing

During the preliminary testing, you will be required to exercise at your maximum level for one to two minutes. By definition, this will feel challenging. However, it should not be any different to your usual training and/or competition demands, and you should recover fully within a 5 minutes period. You will be free to stop the test when you feel it has become too difficult, by raising your hand or mounting the side of the treadmill.

During preliminary testing, we will also collect a very small fingertip blood sample (<1 ml) at the end of each exercise stage. This will be obtained by pricking the finger with a sterile lance and using a small tube to collect the blood sample. This may be associated with minor and very brief discomfort. Whilst there is very small risk of infection with such procedures, this will be minimised via best practice (e.g. the use of an antiseptic wipe, researcher always wearing sterile gloves).

We will also obtain a 4 ml blood sample from a vein in your arm to screen for sickle cell trait (a blood condition known to compromise your oxygen delivery). This will involve piercing the skin with a small sterile needle, and inserting the tip of the needle into a vein in your arm. This can cause minor bruising, and carries a very small risk of infection. However, this is very rare, and is minimised by maintaining best practice. Therefore, all researchers involved in blood sampling will be fully trained in safely collecting and handling blood samples, and signed off as competent by the institutional phlebotomy trainer. At least one researcher present (Oliver Shannon) will have undergone first aid training, in case of emergency. If you have a needle phobia, please discuss this with the lead researcher (Oliver Shannon).

Main trials

The final part of the main trials involves covering 3km as fast as you can. Exercise will be uphill (10 % treadmill gradient) and you will be asked to wear a 10 kg backpack, which we will provide. You will be asked to provide a maximal effort, which will feel difficult. However, again, this should be similar to your usual training and/or competition requirements and you get to choose your exercise speed so that you only go as fast as you feel you can.

Fingertip and venous (8 ml) blood samples will be collected at the start and end of the testing session. The potential risks associated with these techniques are outlined above.

Beetroot juice

Each main trial will be preceded with the consumption of a small amount (140 ml) concentrated beetroot juice. A similar dose has been provided in a number of our previous investigations, and has generally been well tolerated. One commonly reported side effect is a slight pinkish discoloration of the urine and faeces (known as 'beeturia'), which is completely harmless and is caused by the natural red pigments in beetroot.

What happens if something goes wrong?

All of the experimental procedures that will be used in this study have been rigorously tested to ensure that they meet health and safety standards. These are all routine tests and are performed regularly on patients and healthy volunteers. The researchers who perform the tests are all trained and skilled to do so, and at least one researcher will be first aid trained.

In the unlikely event of you experiencing any problems that may be caused by this study you must inform Oliver Shannon immediately (see below) and we will do our utmost to address these issues. Should you be harmed in any way whilst participating in this study, the University maintains clinical trial indemnity insurance. The clinical trial indemnity insurance will only respond in the event that the University is deemed to be legally liable for incidents that occur, as a direct result of the study.

Will my taking part in the study be kept confidential?

All information collected will be kept strictly confidential, other than to those of us who are directly involved with the study within Leeds Beckett University. Any information that leaves Leeds Beckett University will have names and addresses removed so that you cannot be recognised from it. Should any of your blood test results fall outside of the usual range, you will be informed and referred to your GP. The researchers are not medically qualified, and hence will advise you to seek further advice to interpret these results.

Your personal data will be coded at the start of the study by the Investigators who will be the only people able to trace data back to any participant. The Investigator's computers will be in a locked room and password protected. The real names of the individuals will not be used in reports. The data from the study will be kept for 10 years following the completion of the study, thereafter it will be destroyed.

Students studying a Masters degree within the School of Sport will assist with testing as part of their Major Independent Study. They will be provided with a sub-section of the data for their project. This will be anonymous, and will not include participant names.

What will happen to the results of the study?

The findings will form part of a PhD and/or master's student's thesis, and will be written up in these formats. We will also write the study up as a research paper(s) to submit to journals for possible publication. You will not be referred to by your name at any point in published work, only by an anonymous code (e.g. Participant 1). The study findings will be made available to interested participants.

Contact details

Thank you for expressing an interest to participate in this study. If you would like further information, please contact Oliver Shannon, who is organising this study. You may also wish to contact the PhD supervisory team, Prof. John O'Hara (Director of Studies), Dr. Lauren Duckworth (Supervisor), and Dr. Matt Barlow (Supervisor). If you require independent advice about this study at any time during participation, or have a complaint, you may contact Dr. Kevin Deighton (Local Research Ethics Coordinator).

Dr. Kevin Deighton (Local Research Ethics Coordinator)
Senior Lecturer in Sport & Exercise Nutrition
School of Sport, Carnegie faculty
Leeds Beckett University
Headingley campus, Leeds, LS6 3QS
Email: K.Deighton@LeedsBeckett.ac.uk

Oliver Shannon (PhD student)
School of Sport, Carnegie Faculty
Leeds Beckett University
Headingley Campus, Leeds, LS6 3QS
Email: O.Shannon@LeedsBeckett.ac.uk

Prof. John O'Hara (Director of Studies)
Reader in Sport and Exercise Physiology
School of Sport, Carnegie Faculty
Leeds Beckett University
Headingley Campus, Leeds, LS6 3QS
Tel: 0113 8125239 | Email: J.OHara@LeedsBeckett.ac.uk

Dr. Lauren Duckworth
Senior Lecturer in Exercise Physiology
School of Sport, Carnegie faculty
Leeds Beckett University
Headingley campus, Leeds, LS6 3QS
Tel: 01138 126288 | Email: L.Duckworth@LeedsBeckett.ac.uk

Dr. Matthew Barlow
Senior Lecturer in Exercise Physiology
School of Sport, Carnegie faculty
Leeds Beckett University
Headingley campus, Leeds, LS6 3QS
Tel: 0113 8124022 | Email: MatthewBarlow@LeedsBeckett.ac.uk

Consent Form – The effect of beetroot juice on exercise performance, cognitive responses, and physiological functioning at simulated moderate and high altitude
Lead Researcher: Oliver Shannon

1	I understand that all data collected throughout the study will be kept safely and securely, and that my results will remain anonymous.	Yes/No
2	I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason and without affecting my future care.	Yes/No
3	I understand that upon my request any personal data will be removed from the study database should I wish to withdraw my participation, up to the time I complete all the measurements.	Yes/No
4	I consent that my personal data can be retained in a database by the study investigators for the purposes of research and statistical analysis. These data may be made available with my anonymity protected to research students for the purposes of fulfilling their research projects.	Yes/No
5	I understand that the data collected from my participation in this programme can be published in academic/ professional journals, and can also be presented at conferences. My anonymity will be protected at all times and no individual names will be ascribed to any publication.	Yes/No
6	I agree that any personal information about myself will remain locked in a filing cabinet in the Carnegie Centre for Sports Performance and to be destroyed after 10 years of the conclusion of the study.	Yes/No
7	I agree to participate in the project and understand all responsibilities and requirements that are necessary for the study, as detailed in the participant information sheet, which includes visits to Leeds Beckett University and abstaining from alcohol in the 24 hours before testing sessions	Yes/No
8	I understand that in the event of an abnormal result this may be discussed with me and I may be advised to contact my GP	Yes/ No
9	I understand that my medical information will be disclosed to members of the research team to ensure my welfare.	Yes/ No
10	I agree to take part in this study	Yes/ No

Name of Participant (print name) Date Signature

Name of person taking consent Date Signature

School of Sport Screening Questionnaire

Name:_____

Student ID:_____

Risk Factors		Risk Factor	No Risk Factor
Q 1. Age = _____ *years*	Males	**≥ 45**	**< 45**
	Females	**≥ 55**	**< 55**
Q 2. Have any parents, brothers or sisters had a heart attack, bypass surgery, angioplasty, or **sudden death prior to 55 years (male relatives) or 65 years (female relatives)***		**Yes**	**No**
Q 3. Are you currently a smoker – have you quit within the past 6 months – are you exposed to environmental tobacco smoke?		**Yes**	**No**
Q 4. In the past 3 months have you performed at least 30 minutes of moderate intensity physical activity or equivalent on at least 3 days of the week?		**No****	**Yes**
Q 5a. Body mass index = _____ kg.m^{-2} (weight divided by height squared)		**≥ 30**	**< 30**
Q 5b. Waist girth = _____ cm	Males	**> 102**	**≤ 102**
	Females	**> 88**	**≤ 88**
Q 6a. Do you take blood pressure medication		**Yes**	**No**
Q 6b. Resting blood pressure: SBP = _____ *mmHg, DBP* = _____ *mmHg*		**≥ 140/90*****	**< 140/90**
TOTAL NUMBER OF RISK FACTORS * If Yes to early sudden death in family history advise pre-participation screening for SCD ** Avoid maximal testing if currently sedentary ***If BP ≥140/90mmHg treat as High Risk and advise pre-participation screening for SCD			

Signs or Symptoms	S/S	No S/S
Q 9. Do you ever have pain or discomfort in your chest or surrounding areas (neck, jaw, arms or other areas)?	**Yes**	**No**
Q 10. Are you ever short of breath at rest or with mild exertion?	**Yes**	**No**
Q 11. Have you ever experienced dizziness or loss of consciousness during or shortly after exercise?	**Yes**	**No**
Q12. Have you ever been short of breath at rest in the recumbent position or had an attack of breathlessness in the middle of the night which was relieved by sitting up?	**Yes**	**No**
Q 12. Do your ankles ever become swollen (other than as a result of an injury)?	**Yes**	**No**
Q 13. Do you ever have palpitations (=the unpleasant awareness of the heart beating in your chest) or an unusual period of rapid heart rate?	**Yes**	**No**
Q 14. Do you ever suffer from cramp-like pains in your legs, brought on by exertion and relieved after 1-2 minutes of rest?	**Yes**	**No**
Q 15. Has a doctor ever said you have a heart murmur?	**Yes**	**No**
Q 16. Do you feel unusually fatigued or find it difficult to breathe with usual activities?	**Yes**	**No**
SIGNS/SYMPTOMS OF DISEASE	**YES / NO**	

History of Disease	History of Disease	No History of Disease
Q 17. Heart disease	**Yes**	**No**
Q 18. Peripheral vascular disease	**Yes**	**No**
Q 19. Cerebrovascular disease (e.g. stroke)	**Yes**	**No**
Q 20. Chronic obstructive pulmonary disease (emphysema/chronic bronchitis)	**Yes**	**No**
Q 21. Asthma	**Yes**	**No**
Q 22. Interstitial lung disease	**Yes**	**No**
Q 23. Cystic fibrosis	**Yes**	**No**
Q 24. Diabetes mellitus	**Yes**	**No**
Q 25. Thyroid disorder	**Yes**	**No**
Q 26. Renal disease	**Yes**	**No**
Q 27. Liver disease	**Yes**	**No**
HISTORY OF DISEASE	**YES / NO**	

Other conditions	Condition	Condition
Q 28. Do you have any bone or joint problems such as arthritis or a past injury that might get worse with exercise? **(Exercise testing may need delaying or modifying)**	**Yes**	**No**
Q 29. Do you have any other problem that might make it difficult for you to do strenuous exercise? **Details**:	**Yes**	**No**
Q.30 Are you or have you recently been pregnant?	**Yes**	**No**
Q.31 Are you on any prescription medications? **List:**	**Yes**	**No**

	No RF	1RF	1HoD/SS
Total cholesterol = _____ mmol.l^{-1}	**< 5.18**	**≥ 5.18**	
***Or** HDL cholesterol* = _____ mmol.l^{-1}*		**< 1.04**	
Fasting glucose = _____ mmol.l^{-1}	**< 5.55**	**≥ 5.55 ≤ 6.94**	**> 6.94**
***Or** Non-Fasting glucose= _____ mmol.l^{-1}*	**< 7.77**	**≥ 7.77 ≤11.04**	**> 11.04**

*If HDL>1.55 – subtract 1 from the total number of risk factors

Risk Analysis

Total no. of RF (inc blood analysis, if necessary) = _____

SS/HoD = YES / NO – Other conditions to consider: YES / NO

Low risk: No more than one risk factor – safe to do submax/max exercise testing or enter a vigorous programme

Moderate risk: More than one risk factors – safe to do submax exercise testing or enter a moderate programme (max test must be conducted by a person trained in clinical exercise testing)

High risk: One or more signs/symptoms of disease or HoD - cannot do any testing or exercise without physician clearance

Final risk category = _____

- I confirm that the above information which I have provided to Leeds Beckett University is true and accurate to the best of my knowledge and belief and I understand that I must notify promptly of any changes to the information.
- I understand that the information I have provided above may be used as part of an anonymised dataset by staff or students of the School of Sport for completion of coursework or for research or audit purposes (with the appropriate ethical approval in place).

Student Signature:	**Date:**
Assessors Signature:	**Date:**

FOOD AND EXERCISE DIARY

Name

.....

COULD YOU PLEASE FILL IN THE FOLLOWING:-

NAME: ……..

AGE: ……..

WEIGHT: ……..

HEIGHT: ……..

DATE OF BIRTH: ……..

DIETETIC USE ONLY

INSTRUCTIONS

KEEPING A RECORD OF WHAT YOU NORMALLY EAT AND DRINK ENABLES THE DIETICIAN TO CALCULATE YOUR CURRENT DAILY FOOD INTAKE. SO THEREFORE SUBSEQUENT NUTRITIONAL INFORMATION MAY BE TAILORED TO YOUR SPECIFIC REQUIREMENTS

* RECORD **EVERYTHING** YOU EAT AND DRINK OVER A SEVEN DAY PERIOD

* DO NOT ALTER THE FOOD AND FLUID YOU NORMALLY CONSUME

* RECORD THE TIME AT WHICH THE FOOD OR FLUID WAS CONSUMED

* RECORD ALL FOOD EATEN **AS SOON AS POSSIBLE AFTER IT IS CONSUMED**

* IDEALLY AT ALL TIMES THE FOOD RECORD CHARTS SHOULD BE CLOSE AT HAND AS CONTINUAL RELIANCE ON MEMORY INCREASES ERROR

* LIST THE FOODS IN THE ORDER IN WHICH THEY ARE USUALLY EATEN. NOTE BEVERAGES ARE USUALLY STATED LAST AT THE END OF EACH MEAL

* NOBODY IS GOING TO CRITICISE WHAT YOU EAT, SO BE HONEST. DO NOT LEAVE OUT ANY WINE / BEER / SPIRITS OR ANY SWEET / SAVOURY SNACKS, AS THESE WILL CONTRIBUTE SIGNIFICANTLY TO YOUR OVERALL ENERGY INTAKE

* IT IS IMPORTANT TO RECORD ANY **SUPPLEMENTS** TAKEN i.e.: CREATINE, H5, VITAMIN/MINERAL COMPLEXES etc

* RECORD THE AMOUNT i.e. 1 TABLET / 200 MG, AND WHEN YOU TAKE THE SUPPLEMENT IN THE TABLE PROVIDED

INCLUDE AS MUCH DETAIL AS POSSIBLE ABOUT THE FOOD CONSUMED

* IT IS ESSENTIAL TO INCLUDE THE AMOUNTS/PORTION SIZE OF FOOD ACTUALLY EATEN. THIS CAN BE ACHIEVED BY USING HOUSEHOLD MEASURES.

  i.e.: TEASPOON / DESSERT SPOON / TABLESPOON / ICE-CREAM SCOOP
  SMALL / MEDIUM / LARGE PORTIONS / GLASS
  ¼, ½, 1 PINT
  THIN / THICK SPREADING

* IF YOU CANNOT MAKE A RELIABLE ESTIMATE OF A FOOD WEIGHT, DESCRIBE THE FOOD SIZE AS ACCURATELY AS POSSIBLE i.e.: 2 x LOW FAT PORK SAUSAGES 1 INCH DIAMETER, 3 INCHES LONG, RATHER THAN GUESSING THE WEIGHT

* IF CONSUMING PRE-PACKAGED FOODS i.e. PACKET OF CRISPS, IF AT ALL POSSIBLE, TRY TO LOOK AT THE PACKET AND RECORD THE ACTUAL WEIGHT

* RECORD ANYTHING ADDED TO DRINKS e.g. SUGAR, MILK etc

INSTRUCTIONS (CONTINUED)

* **SPECIFY THE TYPE OF FOOD**
  i.e. BREAD: SMALL / MEDIUM / LARGE LOAF
  THIN / MEDIUM / THICK SLICE
  WHITE / BROWN / GRANARY

  MILK: SKIMMED / SEMI-SKIMMED / FULL-FAT
  PASTEURISED / STERILISED / UHT

* IF KNOWN INCLUDE THE BRAND NAME i.e. KELLOGS CORNFLAKES, DEL MONTE FRESH UNSWEETENED ORANGE JUICE

* KEEP FOOD LABELS FOR REFERENCE IF POSSIBLE

* **SPECIFY THE COOKING METHOD**
  IT IS IMPORTANT TO RECORD THE COOKING METHOD AS THIS MAKES A DIFFERENCE TO THE OVERALL ENERGY CONTENT OF THE DIET

  i.e. RAW / BOILED / POACHED / SMOKED / GRILLED / SHALLOW FRIED / DEEP-FRIED / BRAISED / ROASTED

* TRY TO INCLUDE RECIPES FOR HOMEMADE DISHES IF AT ALL POSSIBLE

* IF ONE RECORDS A FOOD ITEM / MEAL BUT ONLY EATS A FRACTION, REMEMBER TO RECORD THE ACTUAL AMOUNT EATEN i.e. ¼, 1/3 etc

* NOTE ANYTHING YOU DO NOT EAT e.g. JACKET POTATO (NOT SKIN), CHICKEN (NOT SKIN)

RECORD ALL EXERCISE UNDERTAKEN:

* RECORD THE TIME YOU CARRY OUT THE EXERCISE

* RECORD THE DURATION OF YOUR EXERCISE SESSION e.g. 60 MINUTES

* SPECIFY THE INTENSITY OF YOUR EXERCISE SESSION:
  e.g. LEVEL 5 ON TREADMILL / 7 MIN PER MILE RUNNING PACE / 6 MPH ON TREADMILL / 15 MPH ON BICYCLE

VERY LIGHT / MODERATE / HARD / VERY HARD
COULD STILL TALK / SLIGHTLY OUT OF BREATH / BREATHING VERY HARD

FOOD RECORD CHART (EXAMPLE)

NAME: ……... DAY: …….

DATE: ……...

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
6:00 am	Del Monte unsweetened fresh orange juice	¼ pint	
	Kellogs Cornflakes	1 x med bowl	
	Granulated sugar	2 x tsp	
	Semi-Skimmed milk (pasteurised)	1 x sm glass	
9:30 am	Streaky bacon	3 x rashers	Grilled
	Pork sausages	2 x (4"x1")	Grilled
	Scrambled egg	med portion	Microwave
	Large, medium sliced wholemeal toast	2 x slices	Toasted
	Thin spreading anchor butter	1 x knob	
	Thin spreading seedless raspberry jam	1 x tsp	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp	
	Granulated sugar	1 x tsp	
Mid Morning			
11 am	McVities large chocolate digestive biscuits	x 3	
	Mug of coffee	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp	
	Granulated sugar	1 x tsp	
Lunch			
1 pm	Heinz country vegetable soup (tinned)	1 x med bowl	
	Crusty granary roll	2 x small	
	Cheese and ham sandwich containing:	x 1	
	-Large, medium sliced white bread	2 x slices	
	-Mild cheddar cheese	1 x matchbox size	
	-Thickly cut honey roast ham	1 x bread size	

	-Hellman's Mayonnaise	1 x tsp	
	Ski Bio (reduced fat) peach/mango yoghurt	1 x 150g pot	
2:30 pm	Banana	1 x med	Raw
	Blackcurrant squash made with soda water	1 x pint	
Mid Afternoon			
4 pm	Packet Seabrook cheese and onion crisps	1 x 30g pack	
	Kit-Kat (4 bar)	x 1	
	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp)	
Dinner			
6pm	Steak and kidney pie (individual, round with puff pastry top)	x 1 (6" x 6")	
	Jacket potato	1 x large	Baked
	Anchor butter	1 x knob	
	Carrots	med portion	Boiled
	Broccoli	med portion	Boiled
	Rhubarb and apple crumble	med portion	
	Bird's custard made with semi-skimmed milk	130 ml	
7:00 pm	Mug of tea	x 1	
	Semi-skimmed milk (pasteurised)	1 x tblsp	
	Granulated sugar	1 x tsp	
Supper			
10:00	Jacob's cream crackers	x 4	
	Mild cheddar cheese	1 x matchbox size	
	Flora Light	1 x knob	
	Mug of Cadbury's chocolate break	1 x 4 tsp	
	Semi-skimmed milk (pasteurised)	1 x tblsp	

SUPPLEMENTS TAKEN (EXAMPLE)

TIME TAKEN	DESCRIPTION	AMOUNT
8:30 am	Vitamin C	1 40mg tablet
9:00 am	Calcium	1 x 500mg tablet

EXERCISE DIARY (EXAMPLE)

TIME	DESCRIPTION	DURATION	INTENSITY
7:30 am	Slow running on the road	45 min	9 min/mile
12:00 pm	**Gym** – cycling warm-up	10 min	12 mph
	Stretching	5 min	Very light
	hack squats – 3 sets of 25 reps		25 kg weight
	Lateral pull-down – 3 sets of 25 reps		10 kg weight
	Hamstring curl – 3 sets of 15 reps		15 kg weight
	Bicep curl – 3 sets of 15 reps		5 kg weight
	Tricep push-down – 3 sets of 15 reps		10 kg weight
	Sit-ups with feet on floor and knees bent – 2 sets of 50		
	Sit-ups with feet on floor, knees to left side of body – 2 sets of 15		
	Sit-ups with feet on floor, knees to right side of body – 2 sets of 15		
	Rowing to cool down	5 min	Light
	Stretching	5 min	Very light
8:30 pm	Running at the track – 800 m warm-up	5 min	10 min/mile
	Stretching	5 min	Very light
	4 x 800m fast with 800m recovery between each set	3 min for fast	Very hard
		set	
		5 min for	Light
		recovery set	
	Stretching	5 min	Very light

FOOD RECORD CHART- Day 1

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 2

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 3

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 4

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 5

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 6

NAME: …….. DAY: …….

DATE: ……..

TIME	***DESCRIPTION OF FOOD OR FLUID***	**WEIGHT OR HOUSEHOLD MEASURE**	**COOKING METHOD**
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

TIME	DESCRIPTION	DURATION	INTENSITY

FOOD RECORD CHART- Day 7

NAME: …….. DAY: …….

DATE: ……..

TIME	*DESCRIPTION OF FOOD OR FLUID*	WEIGHT OR HOUSEHOLD MEASURE	COOKING METHOD
Breakfast			
Mid Morning			
Lunch			
Mid Afternoon			

Dinner			
Supper			

SUPPLEMENTS TAKEN

TIME TAKEN	**DESCRIPTION**	**AMOUNT**

EXERCISE DIARY

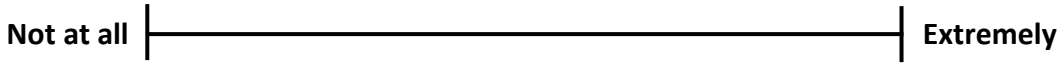
TIME	DESCRIPTION	DURATION	INTENSITY

Ratings of Perceived Exertion (RPE) Scale

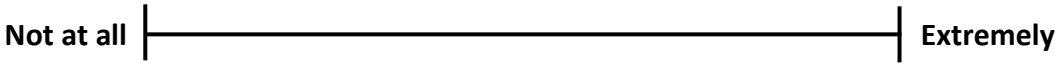
6	
7	**Very, very light**
8	
9	**Very light**
10	
11	**Fairly light**
12	
13	**Somewhat hard**
14	
15	**Hard**
16	
17	**Hard**
18	
19	**Very, very hard**
20	

Please rate how you feel on the following scales, by drawing a vertical line on the following scales….

How mentally fatigued do you feel right now?



How energetic do you feel right now?



BRUNEL MOOD SCALE

Please rate your how you feel using the following questionnaire. You are required to select a number ranging from 0 (not at all) to 4 (extremely), to describe each mood state.

	Not at all	A little	Moderately	Quite a bit	Extremely
1. Active	0	1	2	3	4
2. Alert	0	1	2	3	4
3. Angry	0	1	2	3	4
4. Annoyed	0	1	2	3	4
5. Anxious	0	1	2	3	4
6. Bad tempered	0	1	2	3	4
7. Bitter	0	1	2	3	4
8. Calm	0	1	2	3	4
9. Cheerful	0	1	2	3	4
10. Composed	0	1	2	3	4
11. Confused	0	1	2	3	4
12. Contented	0	1	2	3	4
13. Depressed	0	1	2	3	4
14. Downhearted	0	1	2	3	4
15. Energetic	0	1	2	3	4
16. Exhausted	0	1	2	3	4
17. Happy	0	1	2	3	4
18. Lively	0	1	2	3	4
19. Miserable	0	1	2	3	4
20. Nervous	0	1	2	3	4
21. Panicky	0	1	2	3	4
22. Relaxed	0	1	2	3	4
23. Restful	0	1	2	3	4
24. Satisfied	0	1	2	3	4
25. Sleepy	0	1	2	3	4
26. Tired	0	1	2	3	4
27. Uncertain	0	1	2	3	4
28. Unhappy	0	1	2	3	4
29. Worn-out	0	1	2	3	4
30. Worried	0	1	2	3	4
31. Mixed-up	0	1	2	3	4
32. Muddled	0	1	2	3	4

BRUMS SCORING

SCORING FOR THE BRUMS-32 (Add the responses for the responses to each of the subscales)		
Subscale	**Scores**	**Total**
Anger	ANGRY (3) ___+ ANNOYED (4)___ + BAD TEMPERED (6) ___ + BITTER (7) ___	
Tension	ANXIOUS (5)___+ NERVOUS (20)___ + PANICKY (21)___ + WORRIED (30) ___	
Depression	DEPRESSION (13) ___ + DOWNHEARTED (14) ___ + MISERABLE (19) ___ + UNHAPPY (28) ___	
Vigour	ACTIVE (1) ___+ ALERT (2) ___ + ENERGETIC (15) ___ + LIVELY (18) ___	
Fatigue	EXHAUSTED (16)___ + SLEEPY (25) ___ + TIRED (26) ___ + WORN-OUT (29) ___	
Confusion	CONFUSED (11)___ + UNCERTAIN (27)___ + MIXED-UP (31)___ + MUDDLED (32) ___	
Happy	CHEERFUL (9)___ + CONTENT (12)___ + HAPPY (17) ___+ SATISFIED (24)___	
Calmness	CALM (8) ___+ COMPOSED (10)___ + RELAXED (22)___ + RESTFUL (23)___	

Lake Louise Score (LLS) for the diagnosis of Acute Mountain Sickness (AMS)

A diagnosis of AMS is based on:
1. A rise in altitude within the last 4 days
2. Presence of a headache
PLUS
3. Presence of at least one other symptom
4. A total score of 3 or more from the questions below

SELF-REPORT QUESTIONNAIRE
Add together the individual scores for each symptom to get the total score.

Headache	No headache	0	
	Mild headache	1	
	Moderate headache	2	
	Severe headache, incapacitating	3	
Gastrointestinal symptoms	None	0	
	Poor appetite or nausea	1	
	Moderate nausea &/or vomiting	2	
	Severe nausea &/or vomiting	3	
Fatigue &/or weakness	Not tired or weak	0	
	Mild fatigue/ weakness	1	
	Moderate fatigue/ weakness	2	
	Severe fatigue/ weakness	3	
Dizziness/ light-headedness	Not dizzy	0	
	Mild dizziness	1	
	Moderate dizziness	2	
	Severe dizziness, incapacitating	3	
Difficulty sleeping	Slept as well as usual	0	
	Did not sleep as well as usual	1	
	Woke many times, poor sleep	2	
	Could not sleep at all	3	
	Total score =		

Total score of:
3 to 5 = mild AMS
6 or more = severe AMS

Note:
Do not ascend with symptoms of AMS
Descend if symptoms are not improving or getting worse
Descend if symptoms of HACE or HAPE develop