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Design and conduct of 'Xtreme Alps': A double-blind, randomised controlled study of the effects of dietary nitrate supplementation on acclimatisation to high altitude

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ABSTRACT

The study of healthy human volunteers ascending to high altitude provides a robust model of the complex physiological interplay that emulates human adaptation to hypoxaemia in clinical conditions. Nitric oxide (NO) metabolism may play an important role in both adaptation to high altitude and response to hypoxaemia during critical illness at sea level. Circulating nitrate and nitrite concentrations can be augmented by dietary supplementation and this is associated with improved exercise performance and mitochondrial efficiency. We hypothesised that the administration of a dietary substance (beetroot juice) rich in nitrate would improve oxygen efficiency during exercise at high altitude by enhancing tissue microcirculatory blood flow and oxygenation. Furthermore, nitrate supplementation would lead to measurable increases in NO bioactivity throughout the body.

This methodological manuscript describes the design and conduct of the '*Xtreme Alps*' expedition, a double-blind randomised controlled trial investigating the effects of dietary nitrate supplementation on acclimatisation to hypobaric hypoxia at high altitude in healthy human volunteers. The primary outcome measure was the change in oxygen efficiency during exercise at high altitude between participants allocated to receive nitrate supplementation and those receiving a placebo. A number of secondary measures were recorded, including exercise capacity, peripheral and microcirculatory blood flow and tissue oxygenation.

Results from this study will further elucidate the role of NO in adaption to hypoxaemia and guide clinical trials in critically ill patients. Improved understanding of hypoxaemia in critical illness may provide new therapeutic avenues for interventions that will improve survival in critically ill patients.

1. Introduction

Patients commonly experience hypoxaemia (reduced arterial oxygenation), and none more so than those admitted to critical care units. Surprisingly little is understood about the relationship between arterial oxygenation and survival in the critically ill. Measures to improve patient outcome are

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limited to the continual pursuit of normoxaemia despite the potential hazards associated with this. It is conceivable that alternative strategies could benefit those with hypoxaemia in the setting of established critical illness but the mechanisms governing possible candidate treatments have yet to be elucidated [1]. Cellular and animal models have enhanced our understanding of specific pathways pertinent to hypoxic adaptation but they fail to provide an integrative physiological model for the hypoxaemic critically ill patient.

It has been suggested that the study of healthy human volunteers ascending to high altitude may provide a valid model of the complex physiological interplay that permits human adaptation to hypoxaemia in clinical conditions [2]. Ascent to high altitude is accompanied by a decline in barometric pressure that causes a reduction in the partial pressure of oxygen (PO_2), termed hypobaric hypoxia. The fall in inspired partial pressure of oxygen (PIO_2) leads to a decline in arterial oxygenation at high altitude. Levels of hypoxaemia comparable to those seen in critically ill patients can be mimicked even at moderate altitude, whilst ascent to over 8000 m above sea level results in levels of hypoxaemia that are likely to lie at the limit of human acclimatisation capability [3]. Although such an experimental paradigm is not without limitations [4], it offers a unique insight into human adaptation to hypoxia that cannot be gained from alternative models.

Our first large-scale study of human volunteers at high altitude focussed on determining mechanisms of hypoxic adaptation relevant to the critically ill was carried out in 2007 (Caudwell Xtreme Everest — CXE) [4–6]. Prior to this, many decades of high altitude research expeditions had sought to define distinguishing physiological mechanisms in small groups of selected individuals exposed to hypoxia. In contrast, the *CXE 2007* expedition studied a large group of predominantly altitude naive volunteers who ascended to altitude with an identical ascent profile. In this way, inter-individual differences in performance at altitude could be attributed to underlying responses to the hypoxic challenge. Discrete phenomena could therefore be studied with the ultimate goal of defining a phenotype of successful hypoxic adaptation in order to identify genetic loci and common regulatory/signalling features important for adaptation to hypoxia. This approach has already yielded a number of novel findings that merit further evaluation [1,7–13]. Of specific interest, was the observation that plasma nitrite and nitrate as well as cyclic guanosine monophosphate (cGMP), biomarkers of nitric oxide (NO) production, were elevated on ascent to high altitude and correlated with changes in microcirculatory blood flow [9]. Along with evidence that blood nitrite and nitrate are also elevated in native highland populations [14], this raised important questions relating to the role of nitrogen oxides in the adaptive response to hypoxia. The aim of the '*Xtreme Alps*' study was to explore whether manipulation of NO metabolism and/or availability would lead to alterations in tissue blood flow and oxygenation, and thereby improve tolerance and performance in a hypoxic environment. Such a finding could have implications for the treatment of hypoxaemic critically ill patient.

Hypothesis. The administration of a dietary supplement rich in nitrate will improve oxygen efficiency during exercise (the oxygen consumption (VO_2) to work rate (WR) relationship at steady-state) by augmenting tissue blood flow and oxygenation in the microcirculation. Furthermore, nitrate supplementation will lead to measurable increases in NO bioactivity throughout the body.

2. Materials and methods

The *Xtreme Alps* expedition was a double-blind randomised controlled trial investigating the effects of dietary nitrate supplementation on acclimatisation to hypobaric hypoxia at high altitude in healthy human volunteers. Investigators from the University College London (UCL) Centre for Altitude, Space and Extreme Environment (CASE) Medicine conducted the study, in collaboration with Warwick University. Ethical committee approval was obtained from UCL and the University of Turin.

The primary outcome measurements were a change in oxygen efficiency during exercise at high altitude between the participants allocated to receive nitrate supplementation and those receiving a placebo. Secondary outcome measures included the presence of symptoms of acute mountain sickness(AMS),basic physiological measurements (including oxygen saturation, blood pressure, heart rate and respiratory rate), exercise capacity, microcirculatory blood flow, forearm blood flow, cardiac output, skeletal muscle oxygenation, plasma nitrate and related metabolites, basic lung function and exhaled NO levels.

2.1. Sample size and power calculation

Our primary outcome variable was the change in oxygen efficiency during exercise, which we have previously determined to be 22.3 ($\pm$1.8) % at sea level in a similarly selected group of healthy volunteers [9]. Others have shown that beetroot juice has the ability to improve this efficiency at sea level by 7.1% [15]. Generation of NO via the nitrate–nitrite–NO reduction pathway is expected to be enhanced under conditions of reduced oxygen availability. Thus assuming a greater effect of beetroot juice at altitude compared to sea level, we calculated that in order to demonstrate a 10% improvement in oxygen efficiency 14 subjects would be required in each group, based upon an α level of 0.05 (two-tailed) and β level of 80% (StatMate2, Graphpad software, San Diego, CA).

2.2. Enrolment and screening

Eligible participants were aged $\geq$18 years and not known to be pregnant; anyone deemed unfit to perform exercise at high altitude as previously described [4] was excluded from the study. Participants were recruited using communications sent to recipients of electronic mail from UCL CASE Medicine. These were primarily scientists, medical students and health care workers with an interest in high altitude medicine. A formal health-screening questionnaire was completed prior to acceptance into the study (Appendix A) and reviewed by the expedition medical officer (MK). Anyone deemed to be at risk of health problems at high altitude was assessed in person by the medical officer prior to enrolment.

2.3. Study participants

A total of 28 participants were recruited 21 (75%) of whom were male. Mean age was 28.9 years (21–40), mean weight 73.3 Kg (±11.6), mean height 1.76 m (±0.08); mean BMI 23.6 (±2.69); 3.6% smokers; and 75% had previous exposure to attitude = 3000 m. Subjects were divided into two groups (A and B) according to their availability, and the groups ascended to the Margherita Hut one after the other, with seven days between start of ascent.

2.4. Informed consent

All participants were provided with an information sheet, after which they had the opportunity to ask and have questions answered. If the participant was willing to participate, written informed consent was obtained and a unique identification number was then assigned.

2.5. Randomisation and blinding

Following enrolment participants were randomly allocated to one of two groups, intervention or placebo. We used a custom-designed 2 × 2 randomisation generator (Matlab routine, courtesy of Dr. F. Rigat), which conforms to the CONSORT 2010 guidelines (http://www.consort-statement.org/) and took into account the need for equal numbers of individuals in both groups whilst maintaining gender distribution. Both, participants and investigators were blinded to group allocations for the duration of the study, including the analysis of the primary data sets. During sea-level and high altitude testing group allocation information was kept in a closed envelope solely for access in emergencies; this envelope remained sealed throughout the study.

2.6. Intervention

The intervention was a custom formulated all-natural beetroot/fruit juice blend (produced and generously provided by Aurapa GmbH, Bietigheim-Bissingen, Germany), high in nitrate. The total daily nitrate dose targeted by this intervention was on the same order of magnitude (range 0.10–0.18 mmol/kg/day) as that shown by others to be effective in improving exercise performance at sea level [15,16] and divided into three individual doses for consumption in the morning, at midday and in the evening. The placebo juice was not entirely nitrate-free but contained a significantly (>90%) reduced content, which was achieved by using a beetroot juice in which nitrate was removed using a proprietary selective microbial denitrification method. Administration, at both sea level and high altitude, commenced 72 h prior to testing and continued throughout the study period. In order to account for dietary nitrate intake during the study period, meals were standardised in order to reduce excessive nitrate content. Also, a sample of each course was collected, blended and stored at −40 °C for later nitrate content analysis.

2.7. Study setting

The study took place in August 2010 at the Capanna Regina Margherita ('Margherita Hut'), on the summit of Monte Rosa at 4559 m above sea level. The hut belongs to the Italian Alpine Club, and contains within it a research laboratory that is managed by the University of Turin, Italy.

Baseline measurements were conducted in London at 75 m above sea level: mean barometric pressure 100.5 kPa, mean ambient temperature 24.1 °C and mean oxygen partial pressure 19.7 kPa. Participants then flew to Milan (102 m) and remained there overnight. Ascent began the following day by road to Alagna (1205 m), by gondola and cable car to Punta Indren (3250 m), and finally on foot to the Gnifetti Hut (3611 m). Due to impending severe weather conditions, group A remained for only two nights at 3611 m before further ascent to the Margherita Hut (4559 m), whilst group B ascended after three nights at this altitude. Ascent to the highest altitude was by foot; subjects remained at this altitude for the duration of the study before descending (eight nights for group A, seven for group B) (Fig. 1). In the laboratory at 4559 m the mean barometric pressure was 78.1 kPa, mean ambient temperature 22.6 °C and mean partial pressure of oxygen 15.1 kPa.

2.8. Measurements

All participants had the following measurements taken, both at sea level and at altitude in the Margherita Hut laboratory.

2.9. Nitrate measures

A number of methods were used to measure the concentrations of nitrate, nitrite and other NO metabolites in bodily fluids to obtain information about the absorption, distribution, metabolism and excretion of nitrate, its potential cross-talk with endogenous NO metabolites and effects on pulmonary and renal function.

2.9.1. Plasma nitrite/nitrate concentration

Fasted venous blood samples (5 ml) were taken once at sea level, and on each morning during the study at altitude, in EDTA-containing tubes (BD Vacutainer). This sample was then immediately centrifuged at 800 ×g for 15 min; 1 ml of

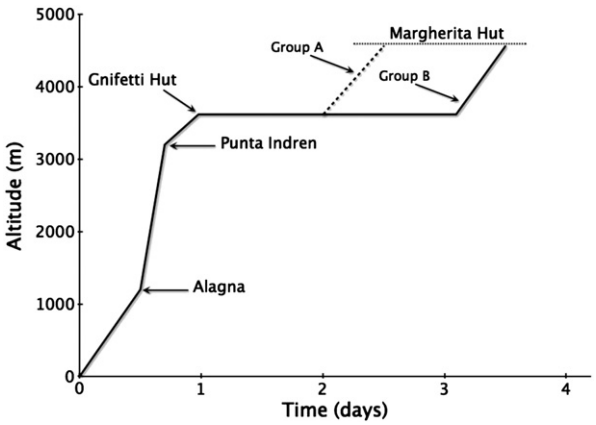

Fig. 1. Ascent profile during the Xtreme Alps expedition to 4559 m. Due to impending severe weather conditions, group A remained for only two nights at 3611 m before further ascent to the Margherita Hut (4559 m), whilst group B ascended after three nights at this altitude.

plasma and the red blood cell pellet were aliquoted into individual cryovials and frozen at −40 °C.

2.9.2. Lung function and exhaled nitric oxide

An ultrasonic spirometer (Easy-One, NDD Medical Technologies, MA, USA) was used to measure standard spirometric variables. Two hand-held spirometric analysis devices connected to gas phase chemiluminescence analysers (CLD88sp with Spiroware software, EcoMedics, Duernten, Switzerland) were used in parallel for breath-to-breath analysis of inhaled and exhaled NO, using individual disposable Spirettes and bacterial filters. Two Denox 88 units (EcoMedics, Duernten, Switzerland) were used to provide NO-free air and enable expiratory flow control according to current American Thoracic Society/European Respiratory Society recommendations for exhaled NO measurements [17]. Both chemiluminescence analysers were equipped with additional stainless-steel bulkhead fittings to supply their ozone generators with pure oxygen from a cylinder (instead of room air) for optimal performance at the reduced pressure at high altitude; devices were calibrated daily with NO (4.00 ppm) and nitrogen (100% N_2 as zero gas) at sea-level as well as at altitude.

2.9.3. Oral nitrate reduction test

The efficiency of nitrate reduction that takes place with commensal oral bacterial flora was assessed by analysis of the conversion of ^{15}N-labelled sodium nitrate into ^{15}N-nitrite [18]. After rinsing the mouth with mineral water, 20 ml of the ^{15}N-labelled nitrate solution was held in the participant's mouth for exactly 3 min before collecting the entire volume. Two 1 ml samples were aliquoted into a cryovial and stored at −40 °C. The test was performed before the mid-day meal, and not directly after ingestion of the beetroot supplement.

2.9.4. Saliva collection

Samples of unstimulated saliva were collected for nitrite and nitrate measurements using dedicated collection devices (Salimetrics Oral Swabs; State College, PA, USA). Swabs were placed under the tongue for 2 min, and then placed into dedicated collection tubes prior to centrifuging at 3000 rpm for 15 min. Two 0.5 ml aliquots of saliva were stored in cryovials at −40 °C before analysis in the UK. Samples were taken just prior to the mid-day meal, and subjects were not allowed to have their teeth brushed shortly before collection, recently consumed their beetroot supplement, or have had recent acute hypoxic exposure.

2.9.5. Exhaled breath condensate collection

Exhaled breath condensate was collected in a non-invasive manner by breathing for 15 min through a mouthpiece connected to a sampling tube contained within an insulated pre-cooled aluminium sleeve (RTube®, Respiratory Research, Inc., Charlottesville, VA, USA). Two 1 ml aliquots of exhaled breath condensate were frozen immediately after collection and kept at −40 °C for later determination of oxidative stress markers and cytokine measurements.

2.9.6. Urine collection

Twenty-four hour urine collections were obtained at sea level and twice at high altitude for calculation of creatinine clearance, nitrite and nitrate clearances and excretion. The collection began following early morning voiding, and precisely 24 h after starting the urine collection the participant urinated for the final time. Urine was collected in 5 litre containers, total volume was determined, after mixing if more than one container was used, and a 15 ml aliquot was frozen at −40 °C. The first sample collection commenced on expedition day five and the second on day eight.

2.10. Metabolic measures

2.10.1. Resting metabolic rate (RMR)

Participants rested in a comfortable, awake state for 45 min whilst their RMR was measured via indirect calorimetry using a validated breath analysis system (Metamax 3b, Cortex, Leipzig, Germany). Measurements of oxygen uptake and carbon dioxide production during the final 15 min of rest were used to determine RMR.

2.10.2. Maximum exercise capacity

A maximal incremental ramp cardiopulmonary exercise test (CPET) was performed on a fully calibrated electromagnetically braked cycle ergometer (Lode Corival, Lode, Groningen, Netherlands) using a breath-by-breath gas-exchange analysis system (Metamax 3b, Cortex, Leipzig, Germany). The guidelines for this protocol had been established from the *CXE 2007* expedition [4] based on the American Thoracic Society/American College of Chest Physicians guidelines for clinical exercise testing [19]. Peak oxygen uptake (VO_2 peak) during the test was selected as the specific measure of exercise performance. This is a common and well validated assessment of fitness used by both exercise physiologists and clinicians [20]. A full calibration of the equipment was performed prior to each test.

2.10.3. Exercise efficiency and economy

Immediately prior to the maximal incremental ramp test, both exercise efficiency and economy were assessed using a constant work rate cycling test. Participants cycled at three steady state work rates (20, 40 and 60 W) below their lactate threshold for 10 min each. The work rates were selected based upon our previous work at altitude when lactate values were measured to ensure that they were below the anaerobic threshold [4]. Exercise economy was defined as the relationship between oxygen consumption and work rate during exercise (ml/min/W), and exercise efficiency was expressed as delta efficiency [4]. Samples of venous blood (5 ml) were taken via an 18G intravenous cannula after 2 min of rest prior to commencing exercise, after minute 8 of each of the three stages of work, and 1 min after cessation of exercise. Two blood samples of 1 ml were aliquoted into cryovials and placed at −40 °C.

2.11. Cardiovascular measures

2.11.1. Cardiac output

At baseline and throughout both above exercise tests, cardiac output was measured using a non-invasive bioreactance system (NICOM; Cheetah Medical, Inc., Vancouver, WA, USA). Bioreactance is based on the principle that electrical impedance across the thorax, resistance to the flow of a high frequency current, is proportional to the amount of blood in the

thorax. The rate of change of impedance with time is therefore related to the rate of change of fluid in the chest over time, which is the blood flow. Because the changes in impedance take place during ventricular contraction, stroke volume can be calculated.

2.11.2. Echocardiography

All study subjects underwent a resting echocardiogram (MicroMaxx, SonoSite, UK) at sea level for the assessment of tricuspid regurgitation (TR). At sea level, participants with known TR underwent estimation of systolic pulmonary artery pressure (SPAP) using a standard echocardiographic technique [21–23]. Volunteers laid in the left lateral position and an apical four-chamber view of the heart was obtained. Doppler ultrasound was used to measure the maximum pressure gradient across the tricuspid valve during systole (ΔP), and SPAP was calculated using an estimated right atrial pressure (RAP) of 5 mm Hg (SPAP = ΔP + RAP). At each time point, SPAP was calculated from the mean of three to five measurements. Images were stored digitally for subsequent analysis.

Measurements were obtained both at sea level, and altitude. At sea level, participants had echocardiograms after breathing room air (21% oxygen), and following exposure to 30 min of hypoxic air (12%). The hypoxic air was delivered using a hypoxic generator (McKinley Altitude Simulator, Higher Peak Performance) via tight fitting facemasks and 12% was selected to emulate the conditions at 4559 m. At altitude this process was reversed: having undergone a resting echocardiogram in room air (12% oxygen), a second echocardiogram was conducted after 30 minute inhalation of 35% oxygen to simulate the sea level partial pressure of oxygen.

2.11.3. Forearm blood flow

Forearm blood flow in the dominant arm was assessed non-invasively using strain gauge venous occlusion plethysmography (EC6 with rapid cuff inflator EC20/AG101 and NIVP3 software; D. E. Hokanson, Inc., Bellevue, WA, USA). A mercury-in-silastic strain gauge of the appropriate size was placed around the maximal circumference of the forearm as the subject rested with their arm elevated to the level of the right atrium. Having occluded the hand circulation using a wrist cuff inflated to 250 mm Hg, a blood pressure cuff placed around the upper arm was inflated to 50 mm Hg, and resting blood flow measurements were taken and oxygen saturation levels recorded. After cuff deflation and 1 min of rest, a standard forearm exercise protocol was performed followed by immediate re-inflation of both cuffs and subsequent recording of circumference changes. The exercise protocol following basal arterial inflow measurements comprised of a series of repeated handgrips of a foam ball for 2 min at a frequency of 0.5 Hz. Measurements at sea level were taken whilst breathing room air (21% oxygen), and following 45 min of breathing a hypoxic gas mixture (12%). As with the echocardiogram, this process was reversed at altitude with 35% oxygen. Total forearm volume was determined for each individual by water displacement to allow for later normalisation of responses.

2.11.4. Skeletal muscle oxygen saturation

A vascular occlusion test was performed with skeletal muscle oxygen saturation (StO_2) measured using near-infrared spectroscopy (NIRS) (InSpectra Model 325; Hutchinson Technology, Hutchinson, MN, USA) in the thenar eminence of the dominant hand. Subjects sat with their arm supported at the level of the right atrium, a probe was applied to their thenar eminence and after a three minute period of stabilisation, a blood pressure cuff was rapidly inflated on the upper arm to 250 mm Hg. After 3 min of arterial occlusion the cuff was rapidly deflated and StO_2 measurements continued for 5 min. Analysis of the rate of muscle desaturation and resaturation provides insights into skeletal muscle oxygen consumption and microvascular reactivity. Measurements at sea level were taken whilst breathing room air (21% oxygen), and following 35 min of breathing a hypoxic gas mixture (12%). As with the echocardiogram and venous plethysmography, this process was reversed at altitude by using 35% oxygen.

2.11.5. Sublingual microcirculation

Whilst at rest and in the supine position, participant's sublingual microcirculation was imaged by sidestream dark field (SDF) imaging using a non-invasive hand held video microscope placed under their tongue (MicroVision Medical, Amsterdam, Netherlands), as previously described [24]. Four 20-second films were recorded from each subject and assessed using the AVA microcirculatory analysis software (MicroVision Medical, Amsterdam, Netherlands).

2.12. Organ function measures

2.12.1. Hepatic metabolism

Changes in global liver function and splanchnic circulation were assessed using a LiMON device (Pulsion Medical Systems, Munich, Germany). This non-invasive device measures the elimination of the diagnostic drug indocyanine green (ICG). As the subject sat at rest, a small dose of ICG (0.25 mg/kg) was injected intravenously and its blood concentration observed over a 15 minute period by transcutaneous pulse densitometry using a probe placed upon the index finger of the non-cannulated hand. ICG elimination kinetics are a function of hepatic biotransformation and splanchnic circulatory blood flow.

2.12.2. Renal function

At sea level and twice at high altitude early morning urine was collected and analysed on site for albumin:creatinine ratio (DCA Vantage Analyzer, Siemens) as a measure of renal protein leak.

2.12.3. Cerebral blood flow and oxygenation

Whilst at rest and having been positioned supine for 10 min subjects had their cerebral blood flow and cerebral tissue oxygen saturations ($StcO_2$) measured. The Fore-sight Absolute Cerebral Oximeter (Casmed, Branford, CT, USA) was used to measure the saturation of cerebral tissue using NIRS probes placed 1.5 cm above the eyebrow overlying the right hemisphere. Cerebral blood flow in the middle cerebral artery was measured via the right temporal window using Transcranial Doppler (MicroMaxx, Sonosite, Bothell, WA, USA) and a 5-1 MHz transducer.

2.12.4. Muscle ultrasound

Assessment of muscle wasting was undertaken whilst at rest with ultrasound (Micromaxx, Sonosite, UK) using a 5 MHz linear array transducer (model HFL38). Limb measurements, in accordance with the protocol described by Campbell and Reid [25,26], were made on the non-dominant side of the subject to identify a mid-point of the limb (bicep, forearm and thigh) with the subject in the supine position. Permanent marker was used to ensure that measurements were made on the same site every time. Three measurements were made using the built-in electronic calliper on a frozen real-time cross-sectional image and averaged. Measurements were taken of the biceps, forearm and thigh muscles. Subjects were measured at sea level and on two occasions at altitude, three days apart.

2.13. Subjective symptom measures

2.13.1. Daily diary

Participants were required to complete a diary of physiological measures and symptoms. Completion of the diary was performed on each morning of the expedition prior to any intake of food (including intervention or placebo juice) or caffeine. The diary included a validated symptom score for AMS; the Lake Louise score [27,28]. Peripheral oxygen saturation (SpO_2), heart rate, blood pressure and respiratory rate were recorded after 5 min of rest. SpO_2, heart rate and respiratory rate were also recorded again after 2 min of controlled step exercise. Finally, any medication prescribed or otherwise taken was recorded in the diary along with alcohol intake. The daily diary entry sheet is shown in Appendix B.

2.14. Additional studies

In addition to the studies mentioned above, a number of subsidiary studies were performed on a separate cohort of subjects not involved in the main randomised controlled trial. Whole body oxygen extraction during exercise at altitude was investigated in five subjects, and the time-course of hypoxic pulmonary hypertension at high altitude was examined in four subjects.

2.15. Sample transport

All samples from the above experiments were transported back to the UK for analysis, with the frozen specimens transported on dry ice. Electronic thermistors measured transportation temperatures throughout.

2.16. Risk management

CASE Medicine has extensive experience with performing research in remote high altitude environments. Initially, the expedition medical officer screened all potential study participants. Written 'Life Threatening Illness' and 'Evacuation' protocols were produced and communicated to all participants. At all times a medical doctor was present in the research laboratory, as was a full medical kit, resuscitation equipment, and supplementary oxygen.

2.17. Analysis plan

In a multi-staged approach to analysis we intend to follow the plan outlined below:

- Subjects, investigators collecting data and those analysing data were all blinded to which group subjects were in (intervention/placebo)
- Entry of individual physiological components into a comprehensive database
- Unblinding of subject cohort allocation
- Comparison of results between intervention and placebo cohorts
- Intention-to-treat analysis of the primary outcome variable: intervention vs. control. Additional analyses of second outcome variables and by intervention received will then be conducted.
- Investigation of the effect of altitude combined with intervention through 2×2 factorial-type analysis of four cohorts (Table 1).

All data will be tested for normality of distribution and transformed/analysed accordingly. Primary outcome measurements of the cohorts outlined in Table 1 will be compared with simple t-tests while secondary outcome (hypothesis-generating) variables will be analysed as deemed most appropriate once the entire data set will be available. Most of the variables will be data on a continuous scale rather than yes/no answers, and may best be analysed by comparing differences rather than absolute values. We will also be interested in exploring the relationship between different sets of variables using multiple linear regression analysis, but realise that the sample size may be insufficient to make adjustments for gender, anthropometric differences, etc.

3. Discussion

This translational study, exploring the efficacy of a dietary supplementation on human acclimatisation to high altitude may have implications for hypoxaemic critically ill patients. The NO pathway offers potential for manipulation in critically ill patients, and the paradigm of healthy volunteers ascending to altitude is a valid testing ground for hypotheses. Results from this study will inform further field studies, more complex mechanistic investigations in hypobaric chambers and clinical trials in the critically ill. It also demonstrates the feasibility of performing complex randomised controlled studies with multiple physiological measures in a hostile, remote environment.

Table 1
The 2×2 factorial analysis of the interaction of intervention and altitude.

	Sea level	Altitude
Intervention	a	b
Placebo	c	d

3.1. Study strengths and weaknesses

This pragmatic but robust approach to investigating potential therapeutic agents in a healthy volunteer model of hypoxaemia will provide data that are difficult to generate through alternative strategies such as animal models and cell culture. It is possible to use a hypobaric chamber to create a hypoxic environment but experiments using this method are limited by cost, tolerability and the risk of decompression illness for investigators who need to frequently enter the chamber. Although effects of nitrate supplementation on skeletal muscle function have been tested in the acute hypoxic setting [16], such effects are likely to differ from those in the more chronic setting, as studied in our field research. Description of integrative physiological changes in human subjects will help determine whether or not this intervention will be a suitable candidate for clinical trials. A double-blind randomised controlled approach to testing the intervention will minimise bias that would otherwise be introduced if other methodologies had been adopted. Strict blinding during data analysis will guarantee that the results will not have been influenced by any pre-conceived ideas held by investigators. Matching ascent profiles amongst the participants allows valid inter-individual comparison of responses to hypoxia, maximising the signal (true physiological differences) to noise (variations in exposure to environmental hypoxia) ratio. Rigorous validation and calibration procedures were followed throughout the study, as per our previously described field study [4].

High altitude is a hostile environment in which factors other than hypoxia may determine direction and quality of the physiological responses observed. These include exertion during exercise during ascent to altitude, environmental temperature, dehydration, intercurrent systemic illness and altitude-related illness. The experiments in this study were therefore conducted in a laboratory with environmental conditions comparable to those experienced at sea level (other than its lower barometric pressure) and after more than 24 h of rest following ascent.

Due to the design of the study, there is a possibility that baseline differences between individuals in the intervention and placebo groups could influence outcome measures. The reason for this is that it was not possible to perform absolute baseline tests on the group prior to randomisation. We were unable to recruit a full participant cohort that was willing to undergo testing at sea level before and after randomisation. There is also potential bias from volunteer self-selection, given that those inclined to climb to high altitude may not be representative of the average population; factors include age and level of physical fitness. Additionally, there were cost implications to participating in the expedition and this may have deterred some volunteers from participating. The small group size also limits any subgroup analysis and risks the possibility of a type II error for some of the measurements taken.

4. Conclusion

This innovative study into the efficacy of oral nitrate supplementation in augmenting human adaptation to hypobaric hypoxaemia may have direct application to clinical medicine. It is hoped that it will ultimately improve and enhance our understanding of hypoxaemic patients and provide new therapeutic avenues for potential interventions to improve survival in the critical care environment.

Competing interests

Xtreme Alps received charitable support from the Friends of University College Hospital NHS Foundation Trust as well as unrestricted research funding from Smiths Medical Ltd. and Deltex Medical Ltd.

Authors' contributions

DM, MG, MM & MF conceived and designed the study; DM, EG, PM, BOF, AC, MK, KM & MF conducted the study; DM, BOF, AC, KM, DL & MF participated in data analysis; the manuscript was written by DM, EG, PM, BOF, MG, DL, MM & MF. All authors contributed to the content of the manuscript. DM was the expedition leader, KM the expedition manager, PM the laboratory manager and MK was the expedition medical officer.

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Sponsors

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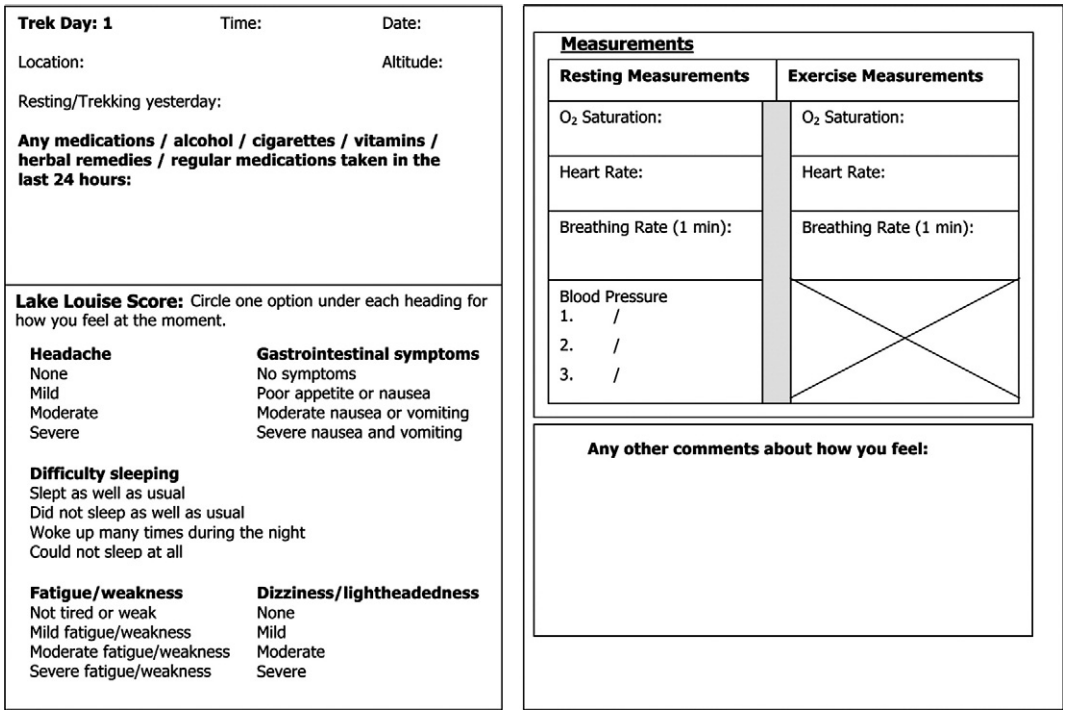
Appendix A. Health Screening Questionnaire sent to all potential subjects

Volunteer name:	
Altitude/travel history	
Have you travelled above 3000 m (9000 ft) before?	Y/N
What is the maximum altitude you have reached previously?	ft/m
For mountaineers, number of ascents above each altitude listed and oxygen use 5000 m 6000 m 7000 m 8000 m	
Have you previously suffered from Acute Mountain Sickness?	Y/N
If yes, please describe details (continue on another sheet if necessary)	
Did you have to descend/abandon a trip to high altitude?	Y/N
Have you previously suffered from HAPE (High altitude pulmonary oedema)?	Y/N
If yes, please describe date, and details	
Have you previously suffered from HACE (High altitude cerebral oedema)?	Y/N
If yes, please describe date, altitude suffered and details	
Have you ever suffered from frostbite, frostnip or cold injury?	Y/N
If yes, please describe details	
Have you travelled abroad in the last year? If so where?	Y/N
Have you ever suffered from medical problems abroad?	Y/N
(If yes give details)	
Volunteer name:	
Medication history	
Are you taking any medication on a regular basis including the oral contraceptive pill?	Y/N
(Please list)	
Have you ever been on any long term medication including psychiatric drugs? Please give details.	Y/N
Are you allergic to any medications?	Y/N
Are you taking any herbal remedies or alternative medicines?	Y/N
(Please list)	
In the last year have you used any recreational drugs? If so what, and how often do you use the drug?	Y/N
Are you taking any vitamin supplements or food supplements?	Y/N
(Please list)	
Do you smoke? (This includes "social smoking")	Y/N
How many manufactured cigarettes per day/week? For how many years?	
How many cigars per day/week? For how many years?	
How much pipe tobacco per week? For how many years?	
How much hand-rolling tobacco per week? For how many years?	
Do you drink alcohol?	
On average, how much do you drink a week? (Give answer in pints/glasses/spirit measures)	
Do you drink coffee regularly	Y/N
Approximately how many cups a day?	
Volunteer name:	
Past medical history	
Have you visited your GP or hospital in the last 12 months for anything apart from a check-up? Please give details and continue on another sheet if required	Y/N
In the past year have you attended hospital or had an operation?	Y/N
Are you currently on the waiting list to see a hospital doctor/specialist for any reason? Please give details.	
Do you suffer from/have you ever had any form of heart disease?	Y/N
Do you suffer from/have you ever had any form of kidney disease?	Y/N
Do you suffer from/have you ever had any form of liver disease?	Y/N
Do you suffer from/have you ever had high blood pressure/hypertension?	Y/N
Do you suffer from/have you ever had any lung disease?	Y/N
Do you suffer from/have you ever had asthma?	Y/N
Do you suffer from/have you ever had epilepsy/blackouts/fits/funny turns	Y/N
Have you ever suffered from a psychiatric or psychological condition, including depression	Y/N
Do you suffer from diabetes?	Y/N
Do you suffer from any allergies?	Y/N
If the answer to any of the above questions is yes, please give details below	

Appendix B. Daily diary completed by all subjects



Trek Day: 1 Time: Date:

Location: Altitude:

Resting/Trekking yesterday:

Any medications / alcohol / cigarettes / vitamins / herbal remedies / regular medications taken in the last 24 hours:

Lake Louise Score: Circle one option under each heading for how you feel at the moment.

Headache	**Gastrointestinal symptoms**
None	No symptoms
Mild	Poor appetite or nausea
Moderate	Moderate nausea or vomiting
Severe	Severe nausea and vomiting

Difficulty sleeping
Slept as well as usual
Did not sleep as well as usual
Woke up many times during the night
Could not sleep at all

Fatigue/weakness	**Dizziness/lightheadedness**
Not tired or weak	None
Mild fatigue/weakness	Mild
Moderate fatigue/weakness	Moderate
Severe fatigue/weakness	Severe

Measurements

Resting Measurements	**Exercise Measurements**
O_2 Saturation:	O_2 Saturation:
Heart Rate:	Heart Rate:
Breathing Rate (1 min):	Breathing Rate (1 min):
Blood Pressure 1. / 2. / 3. /	

Any other comments about how you feel:

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