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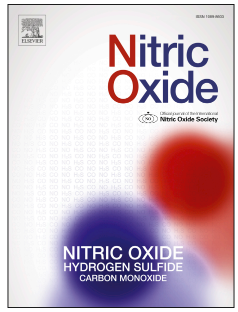

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Effects of dietary nitrate supplementation on symptoms of acute mountain sickness and basic physiological responses in a group of male adolescents during ascent to Mount Everest Base Camp.

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Running head: Effects of nitrate on acute mountain sickness

Key words: Nitrate, beetroot, altitude, acute mountain sickness

Abstract

The purpose of this study was to investigate the effects of dietary nitrate supplementation, in the form of beetroot juice, on acute mountain sickness (AMS) symptoms and physiological responses, in a group of young males trekking to Mount Everest Base Camp (EBC). Forty healthy male students (mean age (SD): 16 (1) yrs) trekked to EBC over 11 days. Following an overnight fast, each morning participants completed the Lake Louise AMS questionnaire and underwent a series of physiological tests: resting blood pressure as well as resting and exercising heart rate, respiratory rate, and peripheral oxygen saturation. The exercise test consisted of a standardised 2-minute stepping protocol and measurements were taken in the last 10 seconds. Participants in the intervention arm of the study consumed 140 ml of concentrated beetroot juice daily, containing approximately 10 mmoles of nitrate, while those in the control arm consumed 140 ml of concentrated blackcurrant cordial with negligible nitrate content. Drinks were taken for the first seven days at high altitude (days 2 to 8), in two equal doses; one with breakfast, and one with the evening meal. Mixed modelling revealed no significant between-groups difference in the incidence of AMS (Odds Ratio – nitrate vs. control: 1.16 (95% CI: 0.59; 2.29)). Physiological changes occurring during ascent to high altitude generally were not significantly different between the two groups (Model Coef (95% CI) – average difference nitrate vs. control: systolic blood pressure, 0.16 (-4.47; 4.79); peripheral oxygen saturation, 0.28 (-0.85; 1.41); heart rate, -0.48 (-8.47; 7.50) (Model Coef (95% CI) – relative difference nitrate vs. control: ventilatory rate, 0.95 (0.82; 1.08)). Modelling revealed that diastolic blood pressure was 3.37 mmHg (0.24; 6.49) higher for participants in the beetroot juice, however this difference was no larger than that found at baseline and no interaction effect was observed. Supplementation with dietary nitrate did not significantly change symptoms of AMS or alter key physiological variables, in a group of adolescent males during a high altitude trekking expedition. There was no evidence of harm from dietary nitrate supplementation in this context. Given the wide confidence intervals in all models, a larger sample size would be required to exclude a false negative

result. Our data suggest that prolonged oral nitrate supplementation is safe and feasible at altitude but has little physiological or clinical effect.

Introduction

Nitric oxide (NO) is a key signalling molecule involved in the regulation of both oxygen delivery and oxygen utilisation through its roles in controlling vascular tone and metabolism [(1)]. In light of this, it is unsurprising that NO is recognised as an important molecule in the processes involved with high altitude acclimatisation and hypoxia (in)tolerance [(2)]. Markers of NO activity in the blood have been found to be; higher in Tibetan natives at 4,200 meters, as compared to American sea-level controls [(3)], increased in lowlanders ascending to high altitude over a number of days [(4)], and associated with altered microcirculatory blood flow [(4)]. Additionally, reduced NO bioavailability is associated with increased pulmonary artery systolic pressures at high altitude [(5)], and inhalation of NO gas (15 ppm) was seen to reduce pulmonary artery pressures in patients suffering from high altitude pulmonary oedema (HAPE) [(6)].

A large proportion of NO is produced by the oxygen (O_2) dependent oxidation of L-arginine. This so called ‘L-arginine-NO pathway’, involves various isoforms of the enzyme nitric oxide synthase [(1)]. In recent years however, it has become apparent that a complementary L-arginine-independent system might also contribute to NO production through the sequential reduction of nitrate to nitrite to NO [(1)]. Nitrate is particularly abundant in foods such as beetroot and spinach [(7)], and following oral consumption, plasma nitrate levels are seen to increase [(7)] and profound effects have been demonstrated on a wide range of human physiological responses including; lowering arterial blood pressure [(8)], lowering resting metabolic rate [(9)], and improving exercise performance [(10)]. Upon acute normobaric hypoxic exposure, nitrate supplementation has also been shown to: increase both peripheral and muscle oxygen saturations during rest and exercise [11], and improve exercise muscle energetics [12] and performance [11; 12; 13]. Conversely, during more sustained hypoxic exposure (a six week normobaric hypoxic training regime), exercise performance was not altered by nitrate supplementation [14]. The only study describing the effects of oral nitrate supplementation on physiological responses to high altitude exposure found a single beetroot juice drink (5 mmoles) restored endothelial function to pre-trek levels following 3 days of trekking from 1523 to 3700 meters [15]. These studies demonstrate that nitrate supplementation can effectively alter physiological responses to acute normobaric and hypobaric hypoxia.

To the authors' knowledge, no literature exists describing the effects of sustained oral nitrate supplementation on the physiological responses to prolonged high altitude exposure, a situation experienced by millions of residents and tourists each year. The aim of this study therefore, was to investigate the effect of oral nitrate supplementation, in the form of beetroot juice, on basic physiological responses and acute mountain sickness symptoms, in a group of young teenaged males trekking to Mount Everest Base Camp (EBC).

Materials and Methods

Study design

The study consisted of a single-blind (participants) parallel group randomised controlled trial, wherein a nitrate supplemented group (n = 21), and a control group (n=19), ascended from Kathmandu (KTM) to EBC over eleven days. During this time period, daily physiological measurements were obtained, and acute mountain sickness symptoms recorded. The investigator was not blinded from the group allocation as during ascent he was required to monitor the prevalence of AMS in each group to ensure the intervention was not causing adverse effects on the participants' health, however, all measurements were taken by trained participants, rather than the investigator, reducing the chance of bias. Randomisation was achieved by using Microsoft Excel to generate a random number list alongside the participants' ID numbers. The participants with the lowest random numbers were allocated to control and the higher numbers to intervention. The study was approved by the research ethics committee at University College London (UCL) in accordance with the declaration of Helsinki (REF: 3198/001).

Participants

Participants were recruited from 45 students who had already agreed to take part in the Bedford School's trek to EBC prior to being approached to participate in the study. As such, the sample size was determined by the number of students that agreed to participate rather than being pre-set using a sample size calculation. Multiple outcomes were considered and the results considered exploratory and interpreted in relation to the precision of any effects to account for potential under-powering. Forty-one healthy male students aged between 14 and 18 were recruited for this study. Following randomisation, one subject withdrew from the trial leaving a total of forty participants. A parent of each student completed a health screening questionnaire on behalf of their child, and this was subsequently screened by a qualified physician, experienced in high altitude medicine, prior to study inclusion. No participants were excluded due to ill health. Both written consent, given by a parent or guardian, and assent, were obtained for/from each student.

Setting and ascent profile

Baseline measurements were made in Bedford, England (50 m – considered to be Sea Level (SL), day 0). Repeat measurements were obtained over the eleven day trek in Nepal at KTM (1300 m, day 1), Monjo (2835 m, day 2), Namche Bazaar (3500 m, days 3, 4, 5), Deboche (3770 m, day 6), Pheriche (4250 m, days 7, 8, 9), Lobuche (4940 m, day 10) and Gorak Shep (5300 m, day 11). On rest days (days 3, 4, 7, 8), all participants remained within 300 vertical metres of the altitude of their overnight residence so as to maintain an identical pattern of hypoxic exposure within this study, and to other studies by our group [16; 17]. One participant did not attend SL testing, and at three locations concerns relating to the health and wellbeing of individual student's, as determined by the trek leader, meant that several participants were deemed 'unable' to trek higher up the mountain and therefore remained at their current location. Accordingly, this meant that these individuals were not able to undergoing testing at all locations. The number of individuals that underwent testing at each location and the reason individuals missed an entire evaluation session (either AMS question or physiological testing) are shown in Supplementary Table 1.

Acute mountain sickness symptoms and physiological measurements

Following an overnight fast, each morning the study participants completed the self-reporting section of the Lake Louise acute mountain sickness (AMS) questionnaire [(18)], and a series of physiological tests [(17)]. The self report section of the Lake Louise questionnaire requires participants to score their symptoms either zero (not present), one (mild), two (moderate) or three (severe) in five different categories (headache; poor appetite, nausea or vomiting; fatigue or weakness; dizziness or light headedness; and difficulty sleeping). The value from each category is summed to give an overall Lake Louise AMS score. An individual was considered to have AMS if they had a Lake Louise score ≥ 3, including a headache and one or more of the other symptoms, along with recent gain in altitude [(18)]. The AMS criterion of a recent gain in altitude was considered to be present from Kathmandu onwards due to the progressive altitude gain profile of the trek. Physiological measurements were acquired immediately before, and in the last 10 seconds of, a standardised 2-minute stepping exercise protocol [(17)]. Those taken prior to the exercise protocol, were preceded by a five minute 'stabilisation period' whereby subjects sat quietly, and were thus considered to be 'resting measurements'. The stepping exercise protocol ('the step test'), consisted of repeatedly stepping on and off a 20 cm step using alternate feet [(17)]. Participants' step rate was set to 1 step (up or down) per second, and this was dictated by an electronic metronome. The measurements taken were resting and exercising; heart rate (Nonin Onyx Vantage 9590, Nonin Medical Inc, Minnesota, USA), ventilatory rate, and peripheral oxygen saturation (SpO_2) (Nonin Onyx Vantage 9590, Nonin

Medical Inc, Minnesota, USA), and resting blood pressure (BP) (in triplicate) (Omron M3, Omron Healthcare, Kyoto, Japan). Participants recorded their measurements in pairs with an investigator available for advice.

Intervention

Participants in the intervention (dietary nitrate supplementation) arm of the study consumed 140 ml of concentrated beetroot juice (Beet it, James White Drinks, Ipswich, UK), containing approximately 10 mmoles of nitrate daily. Those in the control arm consumed the same volume of a concentrated blackcurrant juice cordial (with negligible nitrate content) which was bottled and administered in the same way as the beetroot juice. For logistical reasons we only took sufficient amount of juice for the participants to have seven days of supplementation. As such, drinks were taken for the first seven days at high altitude (days 2 to 8), in two equal doses of 5 mmoles nitrate each; one in the morning with breakfast, and one in the evening with dinner.

Statistical Analysis

Descriptive characteristics are presented as frequencies (i.e., n (%)) for symptoms of AMS (i.e. headache, gastrointestinal symptoms and feelings of fatigue and dizziness, and sleep disorder), and as mean (SD) for physiological measurements (i.e. age, systolic and diastolic blood pressure (SBP and DBP), SpO_2, ventilatory and heart rate). Normality was tested using histograms and P-P plots. Due to low numbers in many categories, individual AMS variables were re-categorised as either present or absent, rather than ranging from zero to three. Logistic mixed regression models were used to evaluate whether beetroot juice was effective in reducing symptoms whilst taking account of repeat measurements at different altitudes within each subject. Results are presented as Odds Ratio (95% Confidence interval; OR (95%CI)). Linear mixed regression models were used to assess whether beetroot juice consumption was related to any of the physiological characteristics measured. Plots of the residuals vs. fitted values were used to evaluate model fit. Skewed residual distribution was found for SpO_2, ventilatory rate and heart rate. For these measurements quantile regression mixed models were also used to further investigate the associations. Results showed similar estimates for SpO_2 and thus results from linear mixed regression models are presented as coefficients (95% CI). For ventilatory rate and heart rate results of linear and quantile regression mixed models were discrepant, thus several transformations were tested. For ventilatory rate log-transformation was found to be the most suitable transformation and log-linear mixed regression models were used. Exponentiated coefficients are given with 95% CI to show relative differences. Package “lme4” [19] was used for both binary and linear models. For heart rate no suitable transformation was found, thus quantile regression mixed models, using the “lqmm” package, were applied [20]. Results for the 50^{th} (median) percentile along with the respective 95%CI’s are shown. All analyses were performed on intention-to-treat basis. Seven participants (control, n=4; intervention, n=3) failed to take at least one juice at one location (n=7), two participants (control, n=1; intervention, n=1) failed at two locations (n=4), and one participant (intervention, n=1) failed at 4 locations (n=4), therefore in total 15 measurements out of the 480 were excluded and per protocol analysis was also performed using those measurement times at which participants complied with the protocol (i.e. 438 data points; 27 data points out of the 480 were also excluded as user missing values). All tests were two-sided and the significance level was set at 0.05. Analyses were performed using R version 2.15.3 [21] incorporating packages as given above.

Results

Sea level comparisons

Forty one adolescents were randomly allocated to either the control (n=20) or intervention group (beetroot juice) (n=21). However, one control participant dropped out thereafter, leaving 19 participants in the control group (mean age (SD): 16 (1) yrs) and 21 participants in the beetroot juice group (mean age (SD): 16 (1) yrs). Participants’ distributions regarding AMS symptoms and other physiological measurements at SL are presented in Tables 1 and 2, respectively. AMS symptoms were most commonly absent or mild (score of 1), and there was no significant difference in distributions between control and beetroot juice (Table 1). Likewise, physiological variables were similar between the control and the beetroot juice groups at sea level, although DBP was on average 5 mmHg higher in the beetroot group (Table 2).

AMS and physiological changes according to altitude and exercise interventions

The trajectories of all AMS and physiological measurements taken at the eight different altitudes and locations (i.e. SL, KTM, Monjo, Namche, Deboche, Pheriche, Lobuche and Gorak Shep) between the two groups are presented in Tables 3 and 4. An altitude effect on the presence of AMS was apparent in both groups showing a tendency to increase with ascent which seemed to normalise when participants' stayed at the same altitude for more than one day (Table 3). This pattern was similar for the physiological measurements with the values being higher, in both groups, after exercise, with the exception of SpO_2 which decreased with altitude and exercise (Table 4).

Effect of beetroot supplementation

Results of mixed modelling reveal that no significant intervention effect of beetroot juice was evident for the presence of AMS (Lake Louise score ≥ 3), or any of its symptoms when taking into account altitude and the day of measurement (Tables 5). Participants in the beetroot juice group had 1.16 (95% CI: 0.59; 2.29) times higher odds of having AMS compared to the control group. This analysis corresponds to the data presented in Table 3 where it can be clearly seen that there is no increased protection/detriment in the beetroot juice group as compared to the control. However, the numbers are small and we cannot discount a halving or doubling of the odds. Results were similar after accounting for altitude and day of measurement. The results in Table 3 illustrates AMS was more likely to occur as participants ascended higher towards EBC, as would be expected, irrespective of study group allocation. In particular, the presence of AMS at SL was 0% in both groups, whilst at the highest altitude, Gorak Shep, the presence of AMS increased to 58% in the control group and 71% in the beetroot juice group, which was a mean difference of 13% (95% CI: -38%; 65%). With respect to individual AMS symptoms, participants in the beetroot juice group had on average non-significantly higher odds of having headache and difficulty sleeping and lower odds of feeling fatigued, having gastrointestinal symptoms, dizziness and/or light-headedness. In all cases results were similar when altitude and day of the measurement were taken into account (*Table 5*). Similar trends with the results of the intention-to-treat analysis were noticed from the results of the per protocol analysis (*data not shown*).

Results evaluating the effects of beetroot juice compared to control on physiological measurements, measured at different altitudes are presented in Tables 4 and 6. Heart rate, SBP, SpO_2, and ventilatory rate were similar between the two groups when taking into account altitude and day of measurement. However, modelling revealed that DBP was increased by 3.37 mmHg (0.24; 6.49) on average for participants in the beetroot juice, as compared to the control group (Table 6). However this difference was noted to be no larger than the difference seen at baseline (Table 4) and so the interaction between intervention and altitude was investigated to see whether there was evidence of the difference increasing over time or remaining at the baseline level. The interaction term (95% CI) in models adjusted for altitude and day was -4.04 (-9.64; 1.56) at Kathmandu and was increased at -2.56 (-8.98; 3.86) at Gorak Shep. The other physiological outcomes were similarly investigated and the interaction (95% CI) at Kathmandu were -6.13 (-12; -0.18), -0.31 (-2.42; 1.80), -3.65 (-12.50; 5.21) and 0.93 (0.81; 1.08) for SBP, SpO_2, heart rate and ventilatory rate respectively. The corresponding values while at Gorak Shep were -2.69 (-9.56; 4.18), -1.36 (-3.74; 1.02), 4.66 (-9.41; 18.74), 1.18 (1.00; 1.38). Although the numbers were small and there was little power to detect interactions, no clear pattern emerged suggesting an interaction effect between the beetroot juice intervention and altitude is unlikely.

Discussion

Main findings

The principle finding of the present study is that nitrate supplementation does not increase or reduce symptoms of AMS, or alter key physiological variables, in a group of adolescent males during ascent to high altitude.

Whilst it is apparent that NO plays an important role in the body's response to hypoxia [1], and numerous studies demonstrated the effects of nitrate supplementation on physiological function, both at SL [10] and in acute normobaric and hypobaric hypoxia [11; 12; 13; 15; 22], our study questions that these effects persist following a more sustained exposure to hypoxia at altitude. We found beetroot juice supplementation, ingested twice daily at moderate to high altitude, had no significant effect on the prevalence of AMS, nor did it significantly affect resting and exercising SpO_2, heart rate, and ventilation rate, and resting blood pressure. Although the sample size was relatively small and the confidence intervals were wide, the point estimates were all close to zero. However, it is possible that this result is a false negative (type 2 error).

There are many factors that differ between the current study and those that have identified physiological alterations following beetroot supplementation in hypoxia, which may account for the discrepancy in findings. The main differences relate to the participants' age, and the length of hypoxic exposure. Firstly, we studied teenagers as opposed to adults, and the effect of dietary nitrate supplementation has been shown to improve skeletal muscle blood flow and vasodilation during hypoxic exercise in older individuals but not in younger people [23]. This suggests that the effect of nitrate supplementation on hypoxic adaptation may be age-dependent.

Secondly, we used a progressive, sustained hypoxic stimulus as opposed to an acute fixed exposure, and the changes in circulating NO activity are known to be dependent on the duration of the hypoxic exposure [2]. Kelly et al. [22] demonstrated that in the absence of beetroot supplementation, plasma nitrite levels were largely unaltered during hypoxic exposure of less than two hours, furthermore, the pulmonary bioavailability of NO has been shown to be reduced 3 hours and 20 hours after arrival at 4559 meters due to an elevation of oxidative stress [5]. Conversely, two studies using very similar ascent profiles to the our current study, found elevated plasma NO metabolite concentrations (including nitrite) after 3-4 days at altitude – values that remained higher for the duration of the study (15 days) [4; 24]. These data suggest that the bioavailability of NO is potentially reduced upon acute exposure to atmospheric hypoxia which may cause a dose of nitrate, acting via the nitrate-nitrite-NO pathway, to have sufficient potency in this situation, explaining the altered hypoxic responses observed over this time frame [11; 12; 13; 22]. Conversely, from 3 to 15 days of progressive hypobaric hypoxic exposure (the time frame of our study), NO bioavailability is in fact elevated compared to the normoxic situation [4; 24], likely due to an upregulation of the NOS-NO system, which may cause any additional NO production via alternative pathways to be less potent, potentially explaining why beetroot supplementation failed to alter the outcomes in the current study. This explanation is not supported by results that found beetroot juice supplementation to improve endothelial function at 3700 meters after 3 days of trekking from lower altitude [15]. An alternative explanation is that dietary nitrate supplementation does not alter any of the specific variables we measured involved with hypoxic acclimatisation; heart rate, blood pressure, SpO_2, and ventilation rate. This is supported somewhat by Casey et al. [23] who found no effect of nitrate supplementation on heart rate, blood pressure, and SPO_2 during very acute (<10 minutes) normobaric hypoxic exposure.

Limitations

A significant limitation of this study was that, secondary to logistical constraints, we were not able to measure circulating NO metabolite concentrations. Accordingly, we are unable to confirm that the dietary nitrate supplementation regime was successful in enhancing NO availability. Previous studies using a similar protocol (2 x 70 ml/day of concentrated beetroot juice produced by the same manufacturer), did find ingestion to increase plasma nitrate and nitrite levels [22], however in these, [22]; however in these, the final dose of beetroot juice was consumed 2.5 hours before blood was taken and physiological variables were measured, potentially ensuring the acute effects of the supplementation were present. In our study, the second beetroot juice of the day was consumed in the evening, with measurements undertaken the following morning approximately twelve hours later. A second limitation was that we did not measure the actual nitrate concentration of beetroot juice utilised in this study, but rather relied on data provided by the manufacturer. Although we have no reason to doubt these data provided by the manufacturer, determining the nitrate content ourselves would have been preferable. In addition, the control drink, whilst looking similar to beetroot juice, did not taste the same and thus the participants were potentially aware of the arm of the study to which they were allocated. There were also a number of missing data points due to individuals not completing parts of the assessment at various locations and not drinking the juice when required. Finally, given the wide confidence intervals that were noticed in all models tested we cannot exclude values that are consistent with a difference being present between the groups. As such, a larger sample size is required to obtain definitive results about the effect of beetroot juice supplementation on the variables measured.

Conclusion

This study found that twice daily supplementation of dietary nitrate (via beetroot juice ingestion) does not reduce or increase the prevalence of symptoms of AMS or alter key physiological variables, in a group of adolescent males during a high altitude trekking expedition when compared to a control group receiving no nitrate supplementation. The study demonstrated that dietary nitrate supplementation at altitude is both feasible and safe. The study was however limited by both its modest sample size and lack of measurement of circulating NO metabolite concentrations. In view of the important role of NO in the physiological response to hypoxia, and given the limitations of this study, these findings should be considered preliminary, and further, adequately powered studies into the efficacy of dietary nitrate supplementation for improving tolerance to high altitude hypoxia are feasible and may be justified.

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Conflict of Interest

None.

Authorship

The authors' contributions are as follows: PJH, KM, E G-K, MF, MPG and DM contributed to the study design, PJH collected the data, PJH, VB, AW and DSM conducted the data analysis. All authors contributed to writing the article, and read and approved the final version of the manuscript.

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Table 1: Prevalence of symptoms of acute mountain sickness (AMS) in the control and the beetroot juice group recorded at sea level.

	Control (n=17)	Beetroot juice (n=18)
Headache		
None	15 (94)	15 (83)
Mild	0 (0)	3 (17)
Moderate	1 (6)	0 (0)
Severe	0 (0)	0 (0)
Gastro-intestinal symptoms		
No symptoms	14 (88)	18 (100)
Poor appetite or nausea	2 (13)	0 (0)
Moderate appetite or nausea	0 (0)	0 (0)
Severe appetite and nausea	0 (0)	0 (0)
Fatigue/weakness		
Not tired or weak	12 (71)	16 (89)
Mid fatigue/ weakness	5 (29)	2 (11)
Moderate fatigue/ weakness	0 (0)	0 (0)
Severe fatigue/ weakness	0 (0)	0 (0)
Dizziness/ light-headedness		
None	13 (81)	18 (100)
Mild	2 (13)	0 (0)
Moderate	1 (6)	0 (0)
Severe	0 (0)	0 (0)
Difficulty sleeping		
Slept well as well as usual	16 (95)	16 (89)
Did not sleep as well as usual	1 (5)	2 (1)
Woke up many times during the night	0 (0)	0 (0)
Could not sleep at all	0 (0)	0 (0)

Results presented as n (%). Two participants from the control group and three from the intervention group did not submit their acute mountain sickness questionnaire at sea level.

Table 2: Summaries of physiological measurements in the control and the beetroot juice group recorded at sea level.

		Control	Beetroot juice	Mean difference (95%CI)
Systolic blood pressure (mmHg)		121 (13)	122 (7)	1 (-5; 8)
Diastolic blood pressure (mmHg)		66 (10)	71 (11)	5 (-2; 11)
SpO_2 (%)				
	Pre-exercise	98 (1)	99 (1)	1 (0; 1)
	Post-exercise	95 (6)	95 (5)	0 (-3; 4)
Heart rate (beats/min)				
	Pre-exercise	70 (11)	69 (6)	-1 (-7; 5)
	Post-exercise	116 (14)	117 (14)	1 (-8; 10)
Ventilatory rate (breaths/min)				
	Pre-exercise	15 (6)	13 (4)	-2 (-5; 1)
	Post-exercise	19 (6)	16 (6)	-3 (-7; 1)

[ʃ]Results are presented as mean (SD). Mean difference is presented along with 95% confidence interval. SpO_2, Peripheral oxygen saturation.

Table 3: Number and percentage of participants reporting presence of acute mountain sickness (AMS) on different days and at the various altitudes within control and beetroot juice groups.

		Acute mountain sickness		
Location and Altitude	Day	Control n/N(%)	Beetroot n/N(%)	%diff (95% CI)
Sea level (50 m)	0	0/17 (0%)	0/18 (0%)	0 (0; 0)
Kathmandu (1300 m)	1	0/19 (0%)	1/21 (5%)	5 (-4; 14)
Monjo (2835 m)	2	4/19 (21%)	3/20 (15%)	-6 (-4; 28)
Namche (3500 m)	3	6/19 (32%)	5/20 (25%)	-7 (-46; 33)
	4	2/19 (11%)	4/21 (19%)	8 (-22; 39)
	5	1/19 (5%)	5/21 (24%)	19 (-1; 47)
Deboche (3770 m)	6	1/19 (5%)	1/21 (5%)	0 (-2; 19)
Pheriche (4250 m)	7	4/19 (21%)	4/20 (20%)	-1 (-37; 35)
	8	1/19 (5%)	0/21 (0%)	-5 (-15; 5)
	9	0/18 (0%)	1/21 (5%)	5 (-4; 14)
Lobuche (4940 m)	10	6/14 (43%)	4/18 (22%)	-21 (-66; 24)
Gorak Shep (5300 m)	11	7/12 (58%)	10/14 (71%)	13 (-38; 65)

Note, the number of participants that reached each location and completed the Lake Louise AMS questionnaire varied over the trek (see Supplementary Table 1 for details).

Table 4: Physiological variables during the trek to Everest Base Camp (EBC) in control and beetroot juice groups.

		Systolic blood pressure (mmHg)		Diastolic blood pressure (mmHg)	
Altitude	Day	Control	Beetroot	Control	Beetroot
Sea level	0	121 (13)	122 (7)	66 (10)	71 (11)
Kathmandu	1	124 (12)	120 (7)	72 (9)	72 (6)
Monjo	2	126 (9)	126 (12)	71 (6)	76 (8)
Namche	3	122 (10)	124 (10)	70 (9)	76 (9)
	4	124 (11)	123 (11)	72 (8)	78 (10)
	5	121 (9)	125 (9)	71 (8)	75 (6)
Deboche	6	124 (10)	125 (9)	71 (8)	77 (8)
Pheriche	7	129 (10)	129 (10)	79 (7)	79 (7)
	8	127 (11)	126 (7)	76 (8)	81 (9)
	9	121 (9)	125 (8)	78 (10)	80 (7)
Lobuche	10	130 (15)	126 (11)	80 (8)	80 (6)
Gorak Shep	11	132 (11)	133 (9)	80 (5)	85 (7)

			SpO_2 pre and post exercise (%)		Heart rate (beats/min)		Ventilatory rate (breaths/min)	
Altitude	Day	Exercise	Control	Beetroot	Control	Beetroot	Control	Beetroot
Sea level	0	Pre-	98 (1)	99 (1)	70 (11)	69 (6)	15 (6)	13 (4)
		Post-	95 (6)	95 (5)	116 (14)	117 (14)	19 (6)	16 (6)
Kathmandu	1	Pre-	97 (2)	97 (1)	75 (11)	74 (9)	15 (5)	11 (3)
		Post-	94 (3)	96 (4)	121 (25)	113 (22)	18 (7)	15 (5)
Monjo	2	Pre-	92 (4)	92 (2)	83 (14)	78 (15)	15 (5)	13 (4)
		Post-	83 (6)	85 (4)	131 (19)	135 (24)	19 (6)	18 (5)
Namche	3	Pre-	89 (5)	89 (3)	82 (13)	84 (14)	14 (5)	13 (4)

		Post-	81 (4)	83 (3)	130 (23)	129 (24)	21 (6)	19 (7)
	4	Pre-	90 (3)	91 (3)	81 (14)	81 (13)	14 (5)	14 (3)
		Post-	83 (4)	84 (5)	124 (25)	126 (23)	20 (5)	20 (5)
	5	Pre-	90 (3)	91 (3)	81 (10)	82 (10)	13 (5)	14 (4)
		Post-	85 (4)	85 (3)	122 (20)	129 (19)	19 (7)	20 (6)
Deboche	6	Pre-	88 (4)	88 (4)	81 (11)	82 (10)	14 (5)	14 (4)
		Post-	81 (4)	83 (3)	125 (31)	133 (19)	20 (6)	19 (7)
Pheriche	7	Pre-	85 (4)	85 (3)	81 (12)	79 (14)	15 (6)	12 (3)
		Post-	79 (3)	81 (4)	124 (23)	128 (17)	21 (6)	18 (6)
	8	Pre-	87 (2)	87 (2)	76 (12)	81 (13)	13 (5)	12 (4)
		Post-	80 (3)	82 (4)	113 (23)	126 (26)	20 (6)	19 (6)
	9	Pre-	89 (3)	88 (3)	82 (14)	85 (12)	13 (4)	12 (3)
		Post-	84 (4)	84 (3)	120 (15)	131 (18)	20 (5)	20 (6)
Lobuche	10	Pre-	82 (3)	82 (4)	88 (13)	91 (11)	14 (5)	14 (4)
		Post-	77 (4)	78 (3)	138 (16)	127 (26)	19 (4)	20 (5)
Gorak Shep	11	Pre-	78 (5)	79 (5)	94 (14)	97 (11)	15 (4)	15 (4)
		Post-	75 (4)	73 (4)	129 (32)	135 (20)	22 (6)	24 (6)

Results are presented as mean (SD). SpO_2, Peripheral oxygen saturation.

Table 5: Modelling results which evaluate the effect of beetroot juice compared to control on the odds of having acute mountain sickness (AMS) or individual AMS symptoms (i.e. headache, gastrointestinal problems, feelings of fatigue, dizziness/ light-headedness, and difficulty sleeping), measured at different altitudes.

	Crude	Adj. for altitude	Adj. for altitude & day
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Acute mountain sickness	1.16 (0.59; 2.30)	1.16 (0.53; 2.54)	1.18 (0.53; 2.64)
Headache	1.09 (0.62; 1.89)	1.09 (0.56; 2.15)	1.10 (0.55; 2.21)
Gastrointestinal problems	0.95 (0.42; 2.12)	0.95 (0.39; 2.29)	0.95 (0.39; 2.32)
Feeling fatigue	0.78 (0.31; 1.99)	0.77 (0.27; 2.19)	0.77 (0.27; 2.21)
Dizziness/ light-headedness	0.78 (0.37; 1.65)	0.75 (0.30; 1.89)	0.75 (0.29; 1.89)
Difficulty sleeping	1.09 (0.62; 1.89)	1.10 (0.56; 2.15)	1.10 (0.55; 2.21)

Odds Ratio, OR.

Table 6: Modelling results which evaluate the effect of beetroot juice compared to control on several physiological measurements, measured at different altitudes during ascent.

	Crude	Adj. for altitude	Adj. for altitude & day
	coef. (95% CI)	coef. (95% CI)	coef. (95% CI)
		Average difference in intervention group vs controls	
Systolic blood pressure (mmHg)	0.16 (-4.47; 4.79)	0.05 (-4.55; 4.65)	0.06 (-4.54; 4.66)
Diastolic blood pressure (mmHg)	3.57 (0.41; 6.72)	3.37 (0.24; 6.49)	3.37 (0.24; 6.49)
SpO_2(%)[a]	0.28 (-0.85; 1.41)	0.51 (-0.50; 1.51)	0.49 (-0.51; 1.50)
Heart rate (beats/min)[a, c]	-0.48 (-8.47; 7.50)	3.99 (-2.61; 10.60)	0.33 (-5.13; 5.79)
		Relative difference in mean of intervention group	
Ventilatory rate[a, b]	0.95 (0.82; 1.08)	0.94 (0.82; 1.08)	0.94 (0.82; 1.08)

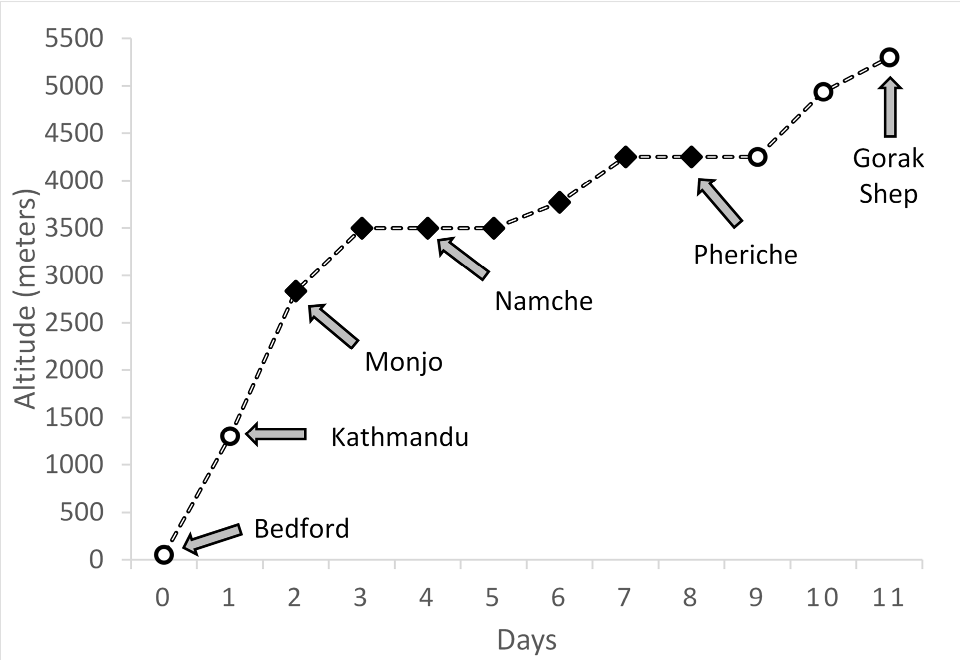
SpO_2, Peripheral oxygen saturation.

[a]Crude models were additionally adjusted for exercise. Further adjustment for altitude and day was also made in subsequent models.

[b]Ventilatory rate was log-transformed. Coefficients of the multilevel model were exponented to give relative differences (i.e. 1.00 represents no mean change) and results are presented as exponentiated regression coefficients (95% confidence interval) and thus units are not required.

[c]Results are based on quantile regression models and are presented as regression coefficients (95% confidence interval) for the 50th percentile (median).

Figure 1. Ascent profile of the study trek.



◆Locations where the supplement was taken, ○Locations where the supplement was not taken

- Oral nitrate supplementation using beetroot juice is safe and feasible at altitude
- Dietary nitrate did not change symptoms of mountain sickness or alter physiology
- These findings are preliminary, and further studies are feasible and justified