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Acute dietary nitrate supplementation improves arterial endothelial function at high altitude: A double-blinded randomized controlled cross over study

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ABSTRACT

Introduction: Dietary nitrate (NO_3^-) supplementation serves as an exogenous source of nitrite (NO_2^-) and nitric oxide (NO) through the $NO_3^- - NO_2^- - NO$ pathway, and may improve vascular functions during normoxia. The effects of NO_3^- supplementation in healthy lowlanders during hypobaric hypoxia are unknown.
Purpose: Determine the effect of acute oral NO_3^- supplementation via beetroot juice (BJ) on endothelial function (flow mediated dilation; FMD) in lowlanders at 3700 m.
Methods: FMD was measured using ultrasound and Doppler in the brachial artery of 11 healthy subjects (4 females, age 25 ± 5 yrs; height 1.8 ± 0.1 m, weight 72 ± 10 kg) sojourning to high altitude. In a randomized, double-blinded crossover study design, FMD was measured 3 h after drinking BJ (5.0 mmol NO_3^-) and placebo (PL; 0.003 mmol NO_3^-) supplementation at 3700 m, with a 24-h wash out period between tests. FMD was also measured without any BJ supplementation pre-trek at 1370 m, after 5 days at 4200 m and upon return to 1370 m after 4 weeks of altitude exposure (above 2500 m). The altitude exposure was interrupted by a decent to lower altitude where subjects spent two nights at 1370 m before returning to altitude again.
Results: Ten subjects completed the NO_3^- supplementation. FMD (mean ± SD) pre-trek value was 6.53 ± 2.32% at 1370 m. At 3700 m FMD was reduced to 3.84 ± 1.31% ($p < 0.01$) after PL supplementation but was normalized after receiving BJ (5.77 ± 1.14% ($p = 1.00$). Eight of the subjects completed the interrupted 4-week altitude stay, and their FMD was lower at 4200 m (FMD 3.04 ± 2.22%) and at post-altitude exposure to 1370 m (FMD 3.91 ± 2.58%) compared to pre-trek FMD at 1370 m.
Conclusion: Acute dietary NO_3^- supplementation may abolish altitude-induced reduction in endothelial function, and can serve as a dietary strategy to ensure peripheral vascular function in lowland subjects entering high altitude environments.

1. Introduction

At high altitude (HA) proper acclimatization is crucial to maintain physiological functions. Preservation of the vascular system is vital for this process, due to its importance for oxygen (O_2) delivery to the tissues and regulation of perfusion pressure to different organs. The definition of a normal vascular response to HA is unclear, and there is no general consensus in the literature of how HA affects

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the arterial endothelial function and vascular tone. Conflicting reports of arterial vasoconstriction or vasodilation upon HA exposure are possibly due to variation in the study designs (population, type and duration of hypoxia) and measurement sites (pulmonary, cerebral, peripheral vasculature) [1,2]. Nevertheless, a key player in obtaining proper endothelial function is nitric oxide (NO). It has been suggested that natives of Tibet living at HA partly have adapted to altitude by NO related mechanisms, and it has been shown that they exhibit high levels of circulating bioactive NO products [4]. Moreover, the combination of high concentrations of circulating bioactive NO products and low hemoglobin is associated with increased forearm blood flow in this population [4]. In native lowlanders, the role of circulating vasoactive nitrogen oxides during acclimatizing to HA was assessed, showing that nitrate (NO_3^-), nitrite (NO_2^-) [5,6] and cyclic guanosine monophosphate (cGMP) [5], biomarkers of NO production and activity, were elevated upon HA acclimatization.

NO is formed through both the L-arginine pathway and the NO_3^--NO_2^--NO pathway. However in an O_2 depleted environment, the L-arginine pathway has a decreased ability to generate NO [8–10], which makes the NO_3^--NO_2^--NO pathway more important. Ingested inorganic NO_3^- is rapidly absorbed from the gut to the blood and then metabolized to NO_2^- by salivary bacteria [9]. This pathway can restore NO production in hypoxic conditions as there is increased generation and use of NO_3^- and NO_2^- [9]. This is due to increased reduction to NO_2^- and NO by deoxyhemoglobin and deoxymyoglobin [11–13], which are more available at HA when blood O_2 saturation is decreased.

At sea level dietary NO_3^- supplementation is effective for optimizing vascular function, resulting in a reduction of blood pressure (BP) [14–17] and improvement of endothelial function with 0.5–4% increase in flow-mediated dilation (FMD) in healthy subjects after acute supplementation [18,19]. Beetroot juice (BJ), known to contain high doses of NO_3^- [17,20], has been demonstrated to have a vasoprotective role [17] and improved FMD after a high fat meal [21]. Based on the positive effect of dietary NO_3^- supplementation on endothelial function at sea level, its' effectiveness in optimizing endothelial function under hypobaric hypoxic conditions should be explored. In this study, the primary aim was to investigate the effects of dietary NO_3^- supplementation on endothelial function during hypobaric hypoxia at HA in lowlanders. The secondary aim was to investigate the effect of prolonged HA exposure on endothelial function.

It was hypothesized that 1) FMD at 3700 m would be depressed compared to pre-trek FMD at 1370 m, 2) that dietary BJ NO_3^- supplementation normalizes FMD at 3700 m, and 3) FMD will remain suppressed compared to pre-trek FMD at 1370 m after a interrupted 4-week exposure to HA (above 2500 m).

2. Materials and methods

This study was performed in Kathmandu and the Rolwaling Valley, Nepal. It was ethically approved by Swedish Research Council and Nepal Health Research Council and performed according to the Helsinki declaration.

2.1. Subjects

A total of 11 healthy lowlanders (4 female, 25 ± 5 yrs, 1.8 ± 0.1 m, 72 ± 10 kg) participated in this field study. The subjects received oral information and read and signed an informed consent. None had been at altitude within 12 months prior to the expedition. A baseline measurement was completed for 10 of the subjects, as one did not follow subject restrictions prior to this measurement. A different subject did not participate in the BJ supplementation due to logistical reasons, 10 subjects completed BJ supplementation. Three subjects did not complete the expedition, rendering a total of 8 subjects for final measurement after 4 weeks at HA.

2.2. Study timeline

FMD was measured 5 times at 3 different altitudes within 39 days of the trekking expedition (Fig. 1). To avoid potential effects of chronobiology, all measurements were obtained in the morning. Pre-trek measure (Test 1) was taken at 1370 m in Kathmandu, one day after arrival. HA was considered as elevations above 2500 m (4 of the 5 weeks of the expedition).

2.2.1. Acute measurements

The NO_3^- supplementation protocol (BJ/placebo (PL)) took place on days 7 and 8 at 3700 m (Test 2/3), after the subjects had spent 3 days walking from 1525 m to 3700 m and after one day residing at 3700 m.

2.2.2. Measurements during altitude exposure

The endothelial function was measured at 4200 m (Test 4) on day 10, after 5 days above 2500 m. Post-HA measures were performed one day after return flight to Kathmandu at 1370 m (Test 5) on day 39 of the trekking expedition, after 4 weeks of HA exposure (above 2500 m) which was interrupted by a decent to lower altitude where subjects spent two nights at 1370 m. Most travel between elevations occurred by walking, with the exception of nine hours bus transportation to the Rolwaling valley (1525 m) on day 2. Additional motorized transportation was used on day 21 (9 h bus from Rolwaling valley) and day 23 (1 h plane to Khumbu valley at 2860 m) due to extreme weather conditions preventing the subjects from climbing a mountain pass between these valleys (two days altitude interruption to 1370 m). The subjects were transported from the Khumbu valley to Kathmandu by plane (1 h) on the final day of the trek (day 38).

2.3. Tests and measurements

FMD was assessed in the brachial artery using a 12-MHz Doppler probe and ultrasound imaging (*Vivid* I, GE Healthcare, USA) following current guidelines [22]. All tests were performed according to a standardized procedure, and consisted of FMD technique to estimate NO-mediated vasodilation, as well as recording of heart rate (HR), BP, and arterial oxygen saturation (SaO_2).

2.3.1. Subject preparation

To avoid measurement bias, subjects avoided caffeine, tobacco and exercise on the testing days and food intake 3 h prior to measurements [22]. Subjects avoided mouthwash and tooth brushing on the BJ/PL test days in order not to wash out lingual bacteria important for NO_3^- reduction [23]. Prior to the measurement, subjects were questioned about their compliance to the study restrictions. In the field subjects were tested inside cabins, and a combination of wood ovens, propane heaters and down sleeping bags were used to control ambient temperature and keep subjects warm (BJ/PL test, room temperature 20 ± 2 °C).

2.3.2. FMD procedure

An occlusion cuff (SC10, D.E. Hokanson Co., Bellevue, USA) was placed on the non-dominant forearm distal to the measuring site and three lead ECG electrodes on shoulders and chest [24]. The measuring site for all FMD measures was above the anticubital fossa, with the arm extended [24]. Once the subject and measurement equipment were prepared, the subjects were requested to