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Effects of oral sodium nitrate on forearm blood flow, oxygenation and exercise performance during acute exposure to hypobaric hypoxia (4300 m)

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ABSTRACT

A reduction in oxygen transport contributes to impaired exercise capacity at high altitude. Since blood flow is mediated, in part, by nitric oxide (NO), we hypothesized that sodium nitrate provided before forearm grip exercise performed at a simulated altitude of 4300 m (hypobaric hypoxia (HH)) would increase forearm blood flow and oxygenation, and decrease the decrement in grip performance. In a double-blind, randomized crossover study, 10 healthy subjects (9 males and 1 female) performed continuous (CGrip) and repeated rhythmic (RGrip) isometric forearm exercise until task failure in normobaric normoxia (NN), 2.5 h following consumption of placebo and sodium nitrate (15 mmol) in HH, and then again post-HH at sea-level pressure. Measurements included forearm blood flow (FBF) and anterior forearm tissue oxygenation (StO_2), mean arterial blood pressure (MAP), arterial blood O_2 saturation (SpO_2), plasma NO reaction products (NO_x) and nitrite, and exhaled NO (PE_{NO}). Compared to baseline testing in NN, performing CGrip and RGrip exercise in HH resulted in significant reductions in forearm blood flow, SaO_2 and StO_2, responses that were accompanied by significant performance decrements (~10%) in both CGrip and RGrip exercise. In spite of a 10-fold increase in plasma NO_x levels and a significant decrease in MAP during CGrip exercise following nitrate consumption, there were no significant main effects of treatment (placebo vs. sodium nitrate) for forearm blood flow, SpO_2, StO_2, or grip performance. PE_{NO} remained unchanged between NN, HH and post-HH conditions with placebo, but increased (~24%) following nitrate supplementation in HH and post-HH. These data do not support a benefit in consuming a single dose of supplemental nitrate on forearm blood flow and isometric exercise in healthy adults at a simulated altitude of 4300 m.

1. Introduction

The role of nitric oxide (NO) in modulating blood flow has been extensively studied since being identified ~20 years ago [1]. NO can be produced from L-arginine and molecular oxygen via NO synthase (NOS), and/or by rapid reduction of NO reaction products (i.e., nitrites and nitrates) [2–5]. A reduction in the partial pressure of oxygen (PO_2) and pH has been proposed to favor the nitrate-nitrite-NO pathway for maintenance of the NO pool [4]. Perhaps this is why Tibetans residing at 4200 m have higher plasma nitrite and nitrate levels, twice the resting forearm blood flow and oxygen delivery, and ~3-fold greater forearm blood flow with exercise compared to U.S. sea level residents [6]. If the Tibetan physiological adaptations are, in part, afforded by NO, then provision of nitrites/nitrates may benefit unacclimatized sea level inhabitants sojourning to high altitude (≥2500 m).

The metabolic demand of working muscle must be met by sufficient oxygen supply. In order to meet oxygen requirements, ventilation increases to elevate the alveolar PO_2 for diffusion into pulmonary capillaries, transport is augmented by cardiac output, and delivery is enhanced by diffusion from active muscle capillaries to mitochondria. While each step is affected when PO_2 is reduced, oxygen transport and/or diffusion from capillary into the muscle is

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believed to limit exercise capacity in hypoxic environments [7–9]. Oxygen transport is dependent upon blood flow and arterial oxygen content (CaO_2), thus improving blood flow to active muscle at altitude could potentially attenuate the decrements in exercise performance.

Undoubtedly, NO contributes to blood flow regulation during exercise performed in hypoxia [10,11]. NO reaction products are significantly reduced in healthy male lowlanders exposed to 3000 m for 24 h [12], thus it is plausible that nitrate supplementation would offset a decrease in NO bioavailability and augment muscle blood flow during exercise performed at high altitude. In support of this hypothesis, Masschelein et al. [13] reported modest increases in muscle oxygenation at rest and during moderate and maximal intensity exercise in normobaric hypoxia (fraction of inspired oxygen (FiO_2) of 11%) following 6 d of nitrate supplementation. Others have also reported improvements in [PCr] recovery rates during exercise and hastened T2* magnetic resonance (MR) signal intensity kinetics (indicative of oxygen delivery) following exercise performed in normobaric hypoxia (FiO_2 of 13–14.5%) after dietary nitrate supplementation [14,15]. Although these investigators did not directly assess blood flow, improved muscle oxygenation, accelerated [PCr] recovery rates and T2* signal intensity are suggestive of enhanced oxygen delivery and/or matching of blood flow to metabolic demands. Since hypobaric hypoxia elicits greater physiological strain than normobaric hypoxia [12,16], the ergogenic potential of nitrates may be much greater when performing work in the former condition.

Whether nitrate supplementation is ergogenic at high altitude currently remains unknown because most of the investigations exploring this question have been conducted in normobaric hypoxia with inconsistent results [11,13–15,17–21]. Presently, to our knowledge only one investigation has examined the ergogenic potential of nitrate supplementation in hypobaric hypoxia (3500 m) with the only significant observation being a reduction in blood lactate at submaximal VO_{2max} (40 and 50%) [22]. Given the paucity of data exploring the benefits of nitrate supplement in hypobaric hypoxia, we sought out to determine the ergogenic potential of oral sodium nitrate supplementation to improve the physiological responses to exercise performed under hypobaric hypoxia conditions in healthy individuals. By employing a moderate to high intensity isometric hand grip exercise protocol and measuring determinants of oxygen transport and tissue oxygenation, we examined the effects of acute nitrate supplementation on forearm exercise performance at 4300 m in a hypobaric chamber. Our hypothesis was that by increasing NO bioactive product availability through acute nitrate supplementation, forearm blood flow and tissue oxygenation will be enhanced and forearm exercise performance at high altitude will be improved.

2. Materials and methods

2.1. Subjects

Ten healthy, recreationally active, adults (9 males, 1 female) with a mean (±SEM) age of 33.9 ± 3.1 years, weight of 85.1 ± 3.7 kg, height of 179.1 ± 3.1 cm, and a body mass index of 26.4 ± 0.7 kg m^{-2} volunteered to participate in this research. All subjects resided at sea level and were unacclimatized to high altitude. Twenty-four hours prior to the experiments, subjects were instructed to avoid high nitrite and nitrate containing foods (provided a list), alcohol, mouthwash, and refrain from exercise. All study participants provided written informed consent prior to completing the study that was approved by the Naval Submarine Medical Research Laboratory's (NSMRL) Institutional Review Board (protocol NSMRL2013.0003) in compliance with all applicable federal regulations governing the protection of human subjects.

2.2. Study design

In a double-blinded randomized-cross over manner, participants completed pre-intervention testing at sea level (baseline), followed by testing in hypobaric hypoxia (4300 m equivalent) and post-hypobaric hypoxia at sea level after consuming a single bolus of 500 mL of a placebo (sodium chloride, 14 mg kg^{-1} body weight) or nitrate (sodium nitrate, 20 mg kg^{-1} body weight, ~15 mmols of nitrate) drink. This dose was selected based on previous research [23], and a pilot study conducted in a subset of subjects (n = 3) that assessed the responses of nitric oxide reaction products (NO_x), nitrites and exhaled nitric oxide (PE_{NO}) over 5 h following ingestion of 500 mL of sodium nitrate (20 mg kg^{-1}). The experimental testing timeline and variables measured are described below and presented in Table 1. On testing days, volunteers arrived at the laboratory between 7:30–8:30 a.m., and a baseline blood sample was obtained from the antecubital vein in lithium heparin and EDTA containing collection tubes. Subsequent samples were obtained in hypobaric hypoxia and post-hypobaric hypoxia. From the lithium heparin sample, blood was immediately analyzed for hemoglobin (Hb) using an i-STAT Handheld blood analyzer (Abbott Laboratories, IL), whereas EDTA collection tubes were immediately centrifuged at 3800 rpm for 15 min at 4 °C, and the plasma was separated and frozen at −80 °C for later analysis of NO reaction products (NO_x) and nitrites. Since there was a significant delay in separating the blood collected in hypobaric hypoxia (~40 min), plasma nitrite levels for this time point are not reported since nitrites are known to undergo rapid reaction with hemoglobin and likely do not reflect values at time of collection when there are significant delays in sample processing [24]. All testing was conducted in the NSMRL Genesis Hyper/Hypobaric chamber with nitrate and placebo experimental sessions separated by a minimum of 1-week. The mean barometric pressure, temperature and relative

Table 1
Experimental testing timeline.

Time (min)	Testing measure/event
Baseline (normobaric normoxia)	
−90	Blood collection
	PE_{NO}
	Continuous [a]BP, pulse, SaO_2
	Resting FBF (Left forearm)
	Maximum voluntary grip strength (left and right)
	CGrip endurance exercise (Left hand)
	Repeat FBF (Left forearm)
	StO_2 (Right forearm)
	10 min forearm occlusion (Right forearm)
	5-min recovery
	RGrip endurance exercise (Right hand)
0	Placebo or Sodium nitrate (15 mmol)
	Travel to 4300 m
+60	Arrive 4300 m
Hypobaric hypoxia	
+84 - +170	[b]Repeat all testing as conducted at baseline
+180	Depart 4300 m
Post-hypobaric hypoxia	
+200 - +260	Repeat all testing as conducted at baseline

PE_{NO}, partial pressure of exhaled NO; BP, blood pressure; SaO_2, arterial blood oxygen saturation; FBF, forearm blood flow; CGrip, continuous static forearm endurance exercise; StO_2, anterior forearm tissue oxygenation; RGrip, rhythmic static forearm endurance exercise.

[a] BP was measured on the non-exercising arm and was switched from the right to left arm after CGrip exercise.

[b] PE_{NO} was determined on 3 occasions (every 30 min) in hypobaric hypoxia. Repeat testing of maximal voluntary grip strength was not performed under hypobaric hypoxia or post-hypobaric hypoxia.

humidity were 760 ± 1 mmHg, 26 ± 0.2 °C and 56 ± 1%, respectively, during baseline normobaric and post-hypobaric hypoxia conditions, and 466 ± 1 mmHg (PO_2 = 98 mmHg), 26 ± 0.2 °C and 58 ± 0.9% during hypobaric hypoxia. Decompression to 4300 m was carried out over 60 min, and recompression to sea level pressure occurred at a decent rate of 305 m/min.

2.3. Heart rate, systemic blood pressure and arterial O_2 saturation

Heart rate and arterial blood oxygen saturation (SaO_2) were measured by ear lobe pulse oximetry, and systemic blood pressure was determined with a finger plethysmograph (BIOPAC® NIBP100D noninvasive blood pressure system) from the non-exercising arm. Parameters were continuously monitored on a laptop computer using the MP150 Data Acquisition system and Acq*Knowledge* Acquisition and Analysis Software version 4.0 (BIOPAC® Systems, Inc., Goleta, CA).

2.4. Forearm blood flow

In a supine position, left forearm blood flow was determined by strain gauge (indium-gallium) plethysmography (Hokanson, Bellevue, WA). The strain gauge was placed around the left forearm distal to the antecubital space (largest circumference), and cuffs were placed around the wrist and upper arm. In a supine position, the subjects arm was fully extended by their side with the forearm raised slightly above the heart. The wrist cuff was inflated to 250 mmHg to exclude hand circulation and the upper arm cuff was inflated to 40 mmHg for 10 s (arterial inflow only), followed by 5 s of deflation (venous emptying) [25]. The mean of ~10 inflation/deflation cycles were used to determine resting blood flow which was measured after a minimum of 5 min of rest in the supine position. Blood flow cannot be assessed during exercise with plethysmography and rapidly drops upon cessation of exercise, thus the mean of the first 2-cuff deflation/pressurization measurements obtained immediately following CGrip exercise were used for analysis.

2.5. Forearm exercise

Prior to consuming the placebo or sodium nitrate drink, the maximal voluntary contraction (MVC) of the left and right forearms were determined using an isometric hand dynamometer (TSD 121C, BIOPAC® Systems, Inc., Goleta, CA) interfaced with a transducer amplifier and the same acquisition system and software used for heart rate, systemic blood pressure and SaO_2. The force output recordings were visible to the subjects on a laptop computer. The highest value obtained from three attempts for each hand was used to determine the initial work load for continuous (CGrip) and rhythmic (RGrip) isometric grip endurance tests. CGrip exercise consisted of left hand grip isometric contractions at 40% MVC until task failure, defined as the time (s) when subjects could no longer maintain grip force within 5% of the target. RGrip exercise was conducted as previously reported with modifications [26]. Using the right hand, subjects performed 2 min stages of rhythmic isometric contractions with a periodicity of 0.5 Hz (i.e., 0.5 s contraction, 0.5 s relaxation) using an audible metronome. Each stage was interspersed with a 60 s rest period. The initial force was set at 40% of MVC with subsequent stages increased by 10% until task failure, i.e., point that subjects grip force fell below 5% of target for >2 consecutive contractions.

2.6. Forearm tissue oxygenation

Tissue oxygenation (StO_2) in the peripheral microcirculation of the right anterior forearm at a depth of 15 mm was determined by securing a self-stick near infrared spectroscopy sensor over the flexor carpi radialis and ulnaris muscles, and continuously measuring the absorbance values between 680 and 800 nm using the Inspectra™ StO_2 Tissue Oxygenation Monitor - Model 650 (Hutchinson Technology Inc., Hutchinson, MN). StO_2 represents the oxygenated Hb (O_2-Hb) relative to the total Hb (deoxygenated Hb (HHb) + O_2-Hb). In order to assess StO_2 during ischemia and recovery from ischemia, arterial blood flow to the right forearm was occluded by inflating a pressure cuff placed around the upper arm to 50 mmHg over each individuals pre-determined occlusion pressure for 10 min to induce localized hypoxia prior to cuff release. Measurement of each subject's minimum occlusion pressure was determined at the beginning of the study by monitoring blood flow in the brachial artery using ultrasound (SonoSite Inc., Bothell, WA) during a gradual occlusion with a blood pressure cuff placed around the upper arm.

The StO_2 data were analyzed using Inspectra™ StO_2 software to calculate resting StO_2 values (mean StO_2 over 5 min prior to conducting each resting blood flow test), the slope of the decline, minimum StO_2, and ischemia area during the 10 min occlusion, and the reactive hyperemia area and peak StO_2 values recorded during recovery from the 10 min forearm occlusion. During the RGrip exercise tests the minima StO_2 was determined for each workload and used for analysis.

2.7. Exhaled nitric oxide

In a seated position, PE_{NO} was measured online with a chemiluminescence NO analyzer (Sievers NOA 280i, GE Analytical Instruments, Boulder, CO) by having the subjects inspire to total lung capacity and exhale against a flow resistor at an expiratory flow rate of 50 mL/s in accordance with guidance provided by the American Thoracic Society (ATS) [27]. During exhalation, the subjects were provided a visual display of the flow output and were required to maintain their expired flow within ±10% of the required 50 mL/s for a minimum of 6 s to obtain a stable NO value. A minimum of 3 measurements that met ATS criteria for exhaled NO were collected [27]. Before each experiment, the span for the NO analyzer was calibrated with a calibration gas containing 45 ppm NO in pure nitrogen, and the zero level was calibrated using NO-free air. The TSI® 400 series mass flow sensor meter (TSI Incorporated, Shoreview, MN) used to monitor the expired flow was calibrated by passing a fixed volume of gas through the sensor using a 3 liter calibrated syringe. The Sievers NO analyzer and TSI® 400 series mass flow sensor were recalibrated at 4300 m for hypobaric hypoxic measurements, and then again upon return to normobaric normoxia. PE_{NO} values are reported as partial pressures in order to compare measurements obtained in normobaric and hypobaric conditions [28,29].

2.8. Plasma NO_x and nitrites

Plasma was filtered through pre-rinsed (4 × with ultrapure water) 10 kDa cutoff filters (EMD Millipore, Billerica, MA) and centrifuged at 14,000 g for 30 min. Total plasma nitrites, nitrates and nitrosothiols (NOx) were determined in 2.5 μL of diluted (1:10) plasma using vanadium (III) chloride reduction and chemiluminescence detection (Sievers Nitric Oxide Analyzer 280i, GE Analytical Instruments, Boulder, CO) as previously described [30]. Plasma nitrites were determined by reducing nitrates to nitrites with nitrate reductase (10 μL of 1U/mL) and NADPH (10 μL of 120 μM) for 1 h at room temperature, reacting with 2,3-diaminonapthalene (10 μL of 316 μM) for 10 min at 24 °C [31], and measuring the fluorescence (excitation 375 nm, emission

450 nm) of 2,3-naphthotriazole (15 μL injection volume) using high-performance liquid chromatography (Waters Separation module e2695, Waters Corp, Milford, MA) with a fluorescence detector (Waters Multi λ Fluorescence Detector, Waters Corp, Milford, MA).

2.9. Calculations

PE_{NO} values were converted from a fractional concentration in ppb to a partial pressure using the following calculation ((barometric pressure − 47 mm Hg) × PE_{NO} (ppb) ÷ 1000) [28]. Subjects were unable to complete all of the RGrip stages so an RGrip endurance index (%RGrip) was calculated as the final workload completed (% MVC) + [(the duration of the final workload attempted (s) ÷ 120) × 10]. Mean arterial blood pressure (MAP) was calculated as ((2 × diastolic blood pressure) + systolic blood pressure) ÷ 3. Vascular resistance was calculated as mean arterial blood pressure ÷ forearm blood flow. CaO_2 was estimated as hemoglobin × SaO_2 X 1.39, and oxygen delivery as arterial oxygen content × forearm blood flow.

2.10. Statistical analysis

Data were analyzed using SigmaPlot 13.0 (Systat Software, San Jose, CA) and SPSS Statistics V22.0 (IBM, Armonk, NY). A one-way repeated measures ANOVA was used to determine differences in the concentration of plasma NO_x, nitrites, and PE_{NO} over 5 h following sodium nitrate supplementation in normobaric normoxia. In order to determine test-retest reliability of left and right forearm exercise prior to beverage consumption, the intraclass correlation coefficient (ICC) was computed using a two factor mixed effects model and type consistency. A two-way repeated measures ANOVA was employed to determine the main effects of treatment (placebo vs. sodium nitrate), condition (normobaric normoxia vs. hypobaric hypoxia and post-hypobaric hypoxia) and the interaction (treatment × condition). When a significant main effect was observed, a Bonferonni *t*-test was performed. In the event that the data failed normality (Shapiro-Wilk) and equal variance (Brown-Forsythe) testing, a simple transformation (log10) was performed and ANOVA testing was repeated. Pearson's correlation coefficients were calculated to determine the association between relative changes in NO_x and plasma nitrites following consumption of the sodium nitrate drink and the relative changes in CGrip and RGrip exercise performance. To account for multiple correlations, the Holm-Bonferroni Sequential Correction Procedure was used [32]. Data are presented as means ± SEM unless otherwise stated. Values of $p < 0.05$ were considered statistically significant.

3. Results

3.1. Plasma NO_x, nitrites and PE_{NO}

During pilot testing under normobaric normoxia in a subset of volunteers ($n = 3$), the mean concentration of plasma NO_x peaked ~13-fold over baseline levels within 60 min of ingestion of sodium nitrate, and remained elevated (>9-fold) over 5 h. Mean plasma nitrite levels more than doubled within 60 min of consuming the sodium nitrate drink, but showed great individual variation in both peak increase (40–240%) and the time course of peak response (60–180 min). Similarly, PE_{NO} levels approximately doubled within 60 min of consuming the sodium nitrate drink but returned to pre-drink PE_{NO} levels between 2.5 h and 3.5 h post-drink.

During the experimental trials, sodium nitrate increased plasma NO_x levels ~10-fold 102 min and 8-fold 206 min following ingestion ($p < 0.001$) (Table 2). Plasma nitrites showed a similar response to that documented in the pilot study, increasing ~2-fold over baseline levels 206 min following consumption of the sodium nitrate solution ($p < 0.01$). In addition to the plasma responses, sodium nitrate increased PE_{NO} (24%) during hypobaric hypoxia testing ($p < 0.001$) and remained significantly elevated during post-altitude testing. In contrast, no changes in PE_{NO} occurred following consumption of the sodium chloride placebo solution.

Table 2
Total plasma NO_x, nitrites and PE_{NO} at baseline in normobaric normoxia, in. hypobaric hypoxia at 4300 m (HH), and post-hypobaric hypoxia (Post-HH).

	Baseline	HH	Post-HH
Plasma NO_x (μM)[a,b,c]			
Placebo	73 ± 8	63 ± 9	65 ± 8
Sodium nitrate	71 ± 7	699 ± 50*†	541 ± 46*†
Plasma Nitrites (nM)[a,b,c]			
Placebo	209 ± 31	NA	226 ± 27
Sodium nitrate	204 ± 24	NA	501 ± 99*†
PE_{NO} (nmHg)[a,b,c]			
Placebo	29 ± 7	26 ± 6	29 ± 8
Sodium nitrate	38 ± 10	47 ± 9*†	43 ± 10†

Data are presented as means ± SEM. NO_x, nitrites + nitrates + S-nitrosothiols. Main effect of [a]condition, [b]treatment and [c]condition x treatment.
*$p < 0.001$ compared to baseline. †$p < 0.01$ compared to placebo within the same condition. NA: Nitrite levels from blood collected in hypobaric hypoxia are not presented since there was a significant time delay before plasma separation.

3.2. Resting physiological parameters

The concentration of hemoglobin did not differ between experimental trials (Table 3). Hypobaric hypoxia resulted in reduced SaO_2 and estimated CaO_2 (−23 ± 2%, $p < 0.001$), and StO_2 (−12 ± 1%, $p < 0.001$), while heart rate increased by 18 ± 3% ($p < 0.001$), changes that were independent of treatment. There was a significant condition × treatment interaction ($p < 0.05$) for MAP, however, post hoc testing only identified a trend for a reduction in MAP in hypobaric hypoxia ($p = 0.074$) with sodium nitrate compared to placebo. Trends for a reduction in MAP and increased forearm blood flow ($p = 0.080$) in hypobaric hypoxia did, however, result in a significant reduction in forearm vascular resistance (−24 ± 5%, $p < 0.05$) and maintenance of oxygen delivery, independent of treatment.

3.3. CGrip and RGrip forearm exercise

A very high degree of reliability was found between MVC tests performed on the left and right hand. The ICC for the left hand was 0.973 (95% CI: 0.893–0.993) and 0.958 (95% CI: 0.831–0.990) for the right hand ($p < 0.001$). There was also a very high degree of reliability between baseline RGrip tests (ICC = 0.919, 95% CI: 0.673–0.980, $p < 0.001$), but low reliability between baseline CGrip tests (ICC = 0.554, 95% CI: −0.795–0.889, $p = 0.122$). There were no differences between baseline CGrip ($p = 0.122$) and RGrip ($p = 0.493$) exercise performed on placebo and sodium nitrate testing days.

Mean CGrip and RGrip exercise performance did not differ between placebo and nitrate trials (Fig. 1A and B), however, there was a mean decrease in CGrip exercise in hypobaric hypoxia (−10 ± 5%) and post-hypobaric hypoxia (−10 ± 4%) ($p < 0.05$), and a 9 ± 2% reduction in RGrip exercise performance in hypobaric hypoxia ($p < 0.001$).

There were no significant correlations between the relative changes in plasma NO_x and nitrites following ingestion of the sodium nitrate drink and the relative changes in CGrip and RGrip exercise performance in hypobaric hypoxia or post-hypobaric

Table 3
Resting physiological parameters at baseline in normobaric normoxia, in hypobaric hypoxia at 4300 m (HH) and post-hypobaric hypoxia at sea level (Post-HH) during the placebo and sodium nitrate trials.

	Placebo			Sodium nitrate		
	Baseline	HH	Post-HH	Baseline	HH	Post-HH
Hemoglobin, g/dL	15.2 ± 0.4	15.0 ± 0.3	15.2 ± 0.3	15.3 ± 0.3	15.0 ± 0.3	15.6 ± 0.3
SaO_2, % of Hb[a]	98.5 ± 0.3	77.3 ± 2.3*	98.7 ± 0.1	98.6 ± 0.2	75.3 ± 2.1*	98.8 ± 0.2
StO_2, % of O_2-Hb/total-Hb[a]	79 ± 1.4	71 ± 1.4*	81 ± 1.4	79 ± 1.6	68 ± 2.2*	80 ± 1.3
CaO_2, mL O_2/g Hb[a]	20.8 ± 0.5	16.1 ± 0.5*	20.8 ± 0.5	21.0 ± 0.4	15.6 ± 0.5*	21.3 ± 0.4
FBF, mL/min/100 mL tissue	3.0 ± 0.3	3.9 ± 0.5	3.2 ± 0.4	2.4 ± 0.3	3.4 ± 0.5	3.0 ± 0.3
Heart rate, beats/min[a]	63 ± 2	75 ± 2*	63 ± 2	62 ± 2	71 ± 3*	63 ± 3
MAP, mmHg	92 ± 3	96 ± 3	89 ± 4	95 ± 2	91 ± 3	93 ± 2
VR, mmHg/blood flow[a]	34 ± 4	28 ± 3*	32 ± 4	46 ± 6	28 ± 4*	35 ± 3
O_2 delivery, mL O_2/min/100 mL tissue	62 ± 6	62 ± 8	67 ± 9	52 ± 8	54 ± 9	63 ± 7

Data are presented as means ± SEM. CaO_2, arterial blood oxygen content; MAP, mean arterial blood pressure; VR, vascular resistance.
[a]Main effect of condition.
*$p < 0.05$ compared to baseline.

hypoxia (data not shown).

3.4. Physiological responses to CGrip exercise

Physiological responses to the CGrip exercise are shown in Table 4. During CGrip exercise performed in hypobaric hypoxia, sodium nitrate resulted in a 7 ± 3% reduction in MAP ($p < 0.01$), whereas heart rate increased by 13 ± 4% with placebo ($p < 0.001$). Independent of treatment, CGrip exercise completed in hypobaric hypoxia led to reductions in SaO_2 (− 17 ± 2%, $p < 0.001$) and blood flow (− 33 ± 8%, $p < 0.05$), and a decrease in oxygen delivery (− 39 ± 7%, $p < 0.01$).

3.5. Physiological responses to RGrip exercise

The mean values for MAP, heart rate, SaO_2 and StO_2 recorded during RGrip exercise up to 60% of MVC are presented in Table 5. There was no treatment effect on any of the parameters measured, however, heart rate was increased and SaO_2 and StO_2 decreased in hypobaric hypoxia at all intensities ($p < 0.05$). Data for intensities ≥70% of MVC are not shown since too few subjects completed these stages during hypobaric hypoxia and analyses could not be performed.

3.6. Effects of forearm ischemia on StO_2

Of the StO_2 parameters examined during (slope of the decline, minimum StO_2 and ischemia area) and following (reactive hyperemia and peak recovery time) brachial arterial occlusion, only a main effect of condition was observed for the minimum StO_2 ($p < 0.05$, data not shown). However, upon post hoc testing there were no significant differences between hypobaric hypoxia and post-hypobaric hypoxia compared to baseline.

4. Discussion

4.1. Principal findings

The main findings from this study is that an acute dose of sodium nitrate does not augment blood flow and oxygen delivery, or static forearm exercise performance in healthy recreationally active adults exposed to a simulated altitude of 4300 m.

NO facilitates hypoxic vasodilation to maintain oxygen delivery, and if the synthesis of NO via the L-arginine–NOS pathway is reduced as can occur in hypoxia [4], consumption of dietary nitrates would provide an immediate source of NO. The dose selected (15 mmol of nitrate) was based on a previous investigation that examined dose-dependent changes in plasma nitrates, nitrites and cGMP following consumption of a single dose of potassium nitrate

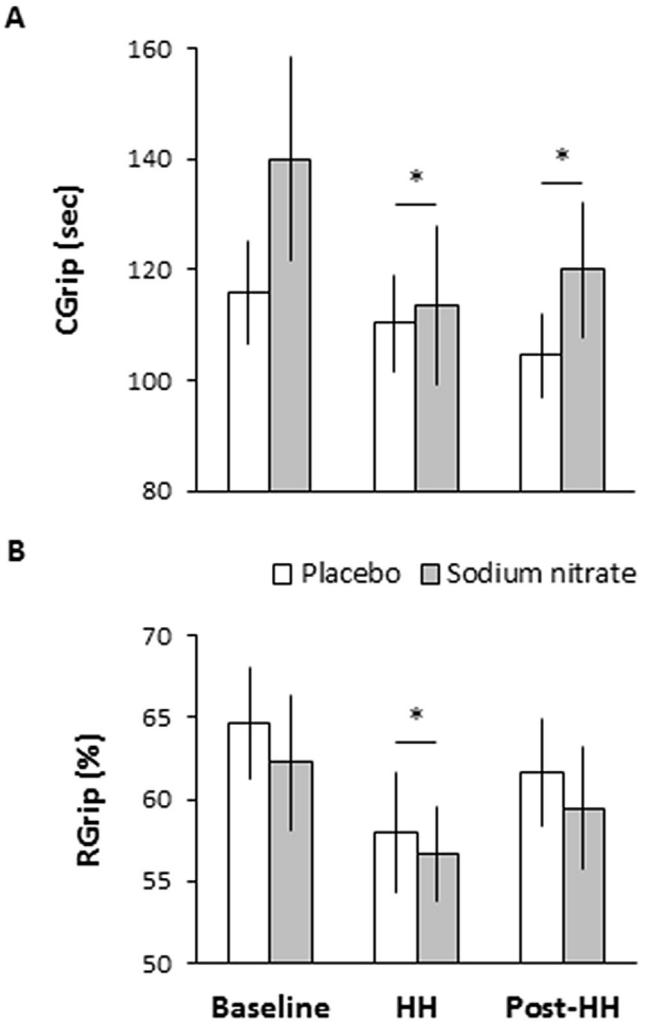

Fig. 1. Forearm grip performance at baseline, in hypobaric hypoxia (HH) and post-hypobaric hypoxia (Post-HH). (A) CGrip endurance exercise performance was determined as the time by which the participants could maintain 40% of their left handed maximal voluntary grip strength. (B) %RGrip endurance exercise performance was calculated as the final workload completed (%) + [(the duration of the final workload attempted (s)/120) X 10]. Data are presented as means ± SEM. *Significantly different ($p < 0.05$) from baseline.

Table 4
Physiological responses to CGrip exercise performed at baseline in normobaric normoxia, in hypobaric hypoxia at 4300 m (HH), and post-hypobaric hypoxia at sea level (Post-HH) during the placebo and sodium nitrate trials.

	Placebo			Sodium nitrate		
	Baseline	HH	Post-HH	Baseline	HH	Post-HH
MAP, mmHg[c]	115 ± 4	115 ± 3[d]	112 ± 3	114 ± 4	107 ± 4[d]	112 ± 2
Heart rate, beats/min[b,c]	89 ± 4	100 ± 4*[d]	89 ± 4	90 ± 4	86 ± 3[d]	86 ± 3
SaO_2, % of Hb[a]	98.4 ± 0.3	82 ± 2.3*	99.0 ± 0.1	98.5 ± 0.2	81.8 ± 1.9*	99.0 ± 0.1
Δ FBF, mL/min/100 mL tissue[a]	4.6 ± 0.6	2.6 ± 0.6*	3.3 ± 0.8	4.0 ± 0.6	3.0 ± 0.6*	3.9 ± 0.6
Δ VR, mmHg/blood flow	−20 ± 5	−9 ± 3	−11 ± 3	−19 ± 6	−20 ± 9	−17 ± 3
Δ O_2 delivery, mL O_2/min/100 mL tissue[a]	94 ± 12	47 ± 10*	68 ± 15	82 ± 11	55 ± 10*	82 ± 12

For MAP, heart rate and SaO_2, data are presented as means ± SEM. For FBF, VR and O_2 delivery, data are presented as the mean changes between pre- and post-CGrip exercise ±SEM.
Main effect of [a]condition, [b]treatment and [c]condition x treatment.
*$p < 0.05$ compared to baseline.
[d]Values sharing similar superscript letters are significantly different, $p < 0.01$.

Table 5
Physiological responses to RGrip exercise performed at baseline in normobaric normoxia, in hypobaric hypoxia at. 4300 m (HH) and post-hypobaric hypoxia at sea level (Post-HH) during the placebo and sodium nitrate trials.

Condition	Placebo			Sodium nitrate		
	Baseline	HH	Post-HH	Baseline	HH	Post-HH
40% MVC						
Completed stage (*n*)	*10*	*10*	*10*	*10*	*10*	*10*
MAP, mmHg	95 ± 4	94 ± 3	94 ± 5	100 ± 3	93 ± 3	93 ± 3
Heart rate, beats/min[a]	74 ± 2	85 ± 4*	76 ± 2	72 ± 3	84 ± 3*	74 ± 3
SaO_2, % of Hb[a]	98.5 ± 0.2	76.3 ± 1.7*	98.7 ± 0.1	97.4 ± 0.6	76.1 ± 1.6*	98.2 ± 0.2
StO_2, % of O_2-Hb/total-Hb[a]	67 ± 2.8	54 ± 4.4*	66 ± 3.1	67 ± 4.0	53 ± 5.1*	66 ± 4.0
50% MVC						
Completed stage (*n*)	*10*	*10*	*10*	*10*	*10*	*10*
MAP, mmHg	103 ± 5	99 ± 4	102 ± 6	104 ± 4	100 ± 3	100 ± 4
Heart rate, beats/min[a]	79 ± 3	91 ± 4*	84 ± 4	81 ± 4	94 ± 5*	81 ± 3
SaO_2, % of Hb[a]	98.8 ± 0.2	78.0 ± 1.9*	98.8 ± 0.2	98.0 ± 0.2	78.9 ± 1.9*	98.5 ± 0.2
StO_2, % of O_2-Hb/total-Hb[a]	62 ± 3.7	50 ± 5.2*	64 ± 2.8	65 ± 4.9	54 ± 4.7*	60 ± 3.1
60% MVC						
Completed stage (*n*)	*9*	*8*	*10*	*8*	*7*	*10*
MAP, mmHg	108 ± 6	101 ± 4	106 ± 6	111 ± 7	104 ± 4	103 ± 4
Heart rate, beats/min[a]	87 ± 4	93 ± 6*	85 ± 3	83 ± 5	100 ± 8*	84 ± 4
SaO_2, % of Hb[a]	98.7 ± 0.2	80.4 ± 2.1*	98.6 ± 0.2	98.3 ± 0.2	81.4 ± 1.9*	98.5 ± 0.1
StO_2, % of O_2-Hb/total-Hb[a]	60 ± 4.8	50 ± 4.6*	62 ± 4.3	63 ± 5.7	51 ± 6.2*	56 ± 4.7

Data are presented as means ± SEM and represent the values obtained during each stage of RGrip exercise up to 60% of MVC. Data for higher workloads (70 and 80% MVC) are not presented because analyses could not be performed as a result of insufficient sample size in HH: 70% MVC stage (n = 3 placebo and n = 1 sodium nitrate), and 80% MVC stage (n = 1 placebo and n = 1 sodium nitrate).
[a]Main effect of condition.
*$p < 0.05$ compared to baseline.

[23]. In this investigation, the authors reported an increase in the concentration of plasma nitrates within 20 min, but a delayed rise in nitrites (1.5 h) that preceded the increase in cGMP [23]. In the current experiment, the mean time that the participants completed CGrip and RGrip exercise testing at 4300 m following sodium nitrate consumption was 2.1 ± 0.03 h and 2.6 ± 0.03 h, respectively, times that did not differ following placebo ingestion. Plasma NO_x and nitrite levels determined during the time course pilot study conducted at sea level, and during the experiment (hypobaric hypoxia and post-hypobaric hypoxia) suggest that the dosing protocol and timing of the physiological tests occurred when the bioavailability of nitrates and nitrites was enhanced from nitrate supplementation. However, it is of note that the coefficient of variation for the plasma nitrite response to sodium nitrate ingestion was 62% and nearly twice the coefficient of variation for plasma nitrate determined at the same time (27%). While plasma nitrates peaked in hypobaric hypoxia and were sustained following return to sea level, the large inter-individual variability in nitrite responses may have resulted in some subjects being tested at a time point when plasma nitrite levels were suboptimal during the sodium nitrate loading trials. Since the primary pathway for dietary nitrate uptake and conversion to nitrite occurs in the oral cavity via the action of commensal bacteria [33], between subject differences in oral microbiome may have contributed to variable nitrite uptake following oral consumption of nitrate.

If NO_x or nitrite bioavailability are primary mediators of the ergogenic potential of nitrate supplementation, then a significant relationship between the relative increase in plasma NO_x or nitrites and the relative changes in CGrip and RGrip exercise tests post-nitrate consumption should have been evident. However, the results from our correlation analyses did not find any significant relationship under hypobaric hypoxia or during normobaric testing. These data suggest that the magnitude of change in plasma nitrites from nitrate supplementation is not a significant mediator of exercise performance, as supported by others [34].

As expected to occur with acute exposure to high altitude, oxygen delivery to the resting forearm was maintained by vasodilation and increased perfusion [35]. With forearm exercise, however, oxygen delivery was reduced by ~40% with CGrip exercise and StO_2 by ~20% with RGrip exercise. These changes were accompanied by significant reductions in CGrip and RGrip endurance exercise performance, suggesting that oxygen transport contributed to

impaired work capacity at high altitude [7,8]. As oxygen extraction was not measured, we cannot exclude the possibility that there was also a limitation in oxygen diffusion. The fact that CGrip endurance exercise remained impaired upon return to sea level pressure does not refute the concept that limited oxygen supply impaired high altitude performance, and instead, may be more related to pain and fatigue and/or the lower reliability of this test compared to the RGrip test [36]. Nonetheless, nitrate supplementation offered no benefit to offset the effects of hypobaric hypoxia on oxygen delivery and tissue oxygenation during exercise.

Investigators that have examined the ergogenic properties of nitrate supplementation in hypoxia (FiO_2 range: 11–15%), and reported improvements that could be attributed to oxygen transfer and consumption, have examined large muscle groups involving bilateral leg exercise including knee-extension [15], cycling [13,19,20] and running [21] performance tests. From these studies, subtle increases in SaO_2 (~2–3%) and leg (*vastus lateralis*) StO_2 (~3–5%), modest improvements in $\dot{V}O_2$ kinetics (~29%) and PCr recovery kinetics (16%), and reduced submaximal $\dot{V}O_2$ contributed to faster time trials [20,21] and extended time to exhaustion [13,15]. The primary differences between these investigations and ours are the size of the active muscle mass and duration of exercise protocol. The lower body comprises ~40% of total body mass, whereas a hand and forearm comprises ~4% [37]. The significance is that exercising a smaller muscle mass reduces the hypoxic effects on pulmonary and muscle $\dot{V}O_{2peak}$ as a result of improved pulmonary gas exchange and a decrease in the rightward shift in the O_2-Hb dissociation curve resulting in increased SaO_2, and thus, CaO_2 and oxygen delivery [7]. While a lower physiological strain associated with activation of a small muscle mass would seem to reduce the ergogenic potential of nitrate supplementation for forearm exercise, this is unlikely as oxygen transport and work capacity (grip endurance) are still impaired with acute hypoxic exposure, as demonstrated herein. The duration of the exercise protocol, however, may explain the disparate results. The mean duration of CGrip and RGrip exercise tests in hypobaric hypoxia was just under 2 min and 2 min stages, respectively, compared to the longer mean exercise duration of 6–~30 min where ergogenic benefits with nitrate supplementation where previously documented [13,15,19–21]. In support of this, Kelly et al. [38] reported extended time to exhaustion (cycling) with daily beetroot juice in normobaric normoxia when the duration of the tests were 4–12 min (intensity dependent, 60–80%), but only a trend when the test was ~3 min (100% peak power). While this explanation is plausible, the significant differences in the physiological responses to exercise between normoxia and hypoxia do not allow for data extrapolation. Moreover, the fact that other investigators who observed no ergogenic effects of nitrate supplementation on running and cycling performance of longer duration (~5–45 min) in normobaric hypoxia [17] and hypobaric hypoxia [22] concurs with the current findings and renders the above latter explanation unlikely.

An alternative reason for not observing an effect with sodium nitrate is that the pool of NO was sufficient to mediate hypoxic vascular responses in younger adults [18]. The major source of NO in hypoxia is believed to be from nitrite reduction, or release from nitroso storage pools because global NOS inhibition does not reverse hypoxic induced vasodilation [39]. Upon exposure to high altitude (3520 m), eNOS expression does in fact decrease over the first 4 days, while nitrites/nitrates concomitantly increase [40]. The fact that the concentration of plasma NO_x did not decrease with placebo, or that there were no differences in StO_2 following brachial arterial occlusion between placebo and nitrate supplementation suggests that NO metabolism was not significantly altered and a longer hypoxic exposure time may be required to impact NO mediated vascular control. For instance, when a single dose of nitrate (5.0 mmol) was supplemented on days 7 or 8 at 3700 m during a 39 d mountain expedition, flow mediated dilation was similar to sea level values with nitrate supplementation, but significantly impaired with placebo [41]. We, therefore, cannot exclude the possibility of vascular benefits with nitrate supplementation when the duration of exposure to high altitude is several days to weeks.

Although nitrate supplementation has been reported to offer no benefit in reducing symptoms of acute mountain sickness or increasing SaO_2 during a sojourn from 2835 m to 4250 m [42], benefits may still exist. In contrast to systemic compensatory vasodilation, the arterioles within the pulmonary circulation constrict during hypoxia causing pulmonary arterial pressure to increase that may subsequently lead to high-altitude pulmonary edema (HAPE) [43]. Increased pulmonary arterial pressures at high altitude have been reported to be accompanied by an increase in transpulmonary endothelin-1 with a concomitant decrease in nitrites [44]. In addition, an inverse relationship between pulmonary arterial pressure and PE_{NO} has been reported in mountaineers exposed to 4559 m from 2 to 48 h, and those HAPE-susceptible exhibited the highest pulmonary arterial pressures and lowest PE_{NO} [45,46]. In the present investigation, nitrate supplementation resulted in a significant increase in PE_{NO} in hypobaric hypoxia. While PE_{NO} and circulating plasma NO_x were not significantly altered in hypobaric hypoxia with placebo during the current acute altitude exposure, others have reported gradual declines in PE_{NO} during the first 3 d at 3800 m and a substantial decline in plasma NO reaction products following 24 h at 3000 m [12,47]. Given nitrate supplementation led to significant increases in plasma NO_x, nitrites and PE_{NO} in the current investigation, there may be value in exploring this strategy to prevent, or reverse, the decline in PE_{NO} and circulating NO_x that occurs with more prolonged altitude exposures to help mitigate hypoxic increases in pulmonary arterial pressure.

We acknowledge that exhaled NO levels do not reflect pulmonary vascular function, yet an increase in upper airway NO production may increase axial back diffusion of NO into the alveoli and circulation that could offer protection from developing pulmonary hypertension. Although researchers have suggested that changes in pulmonary NO flux, as indicated from exhaled NO measurements, do not appear to significantly contribute to the development of pulmonary hypertension during ascent to high altitude [48], the efficacy of nitrate supplementation in reducing pulmonary arterial pressure remains to be fully explored. In light of not measuring pulmonary arterial pressure, this remains a speculative, yet an attractive hypothesis for future research.

4.2. Study limitations and strengths

There were methodological limitations within the present study that deserve mention. Because two different exercise tests were performed within a very narrow window, we could not measure blood flow and StO_2 with both CGrip and RGrip exercise protocols. The strain gauge, wrist and upper arm cuffs had to be fitted to a single arm so that measurements could be made immediately prior to and following CGrip exercise. In addition, the StO_2 sensor was secured to the alternate anterior forearm so that ischemia and RGrip exercise measurements could be performed just after the cessation of forearm blood flow measurements. These methodological challenges prevent us from concluding that nitrate supplementation did not improve StO_2 during CGrip exercise and forearm blood flow with RGrip exercise. This, however, is very unlikely since an increase in delivery would yield an increase in microvascular oxygenation. A major strength of this study is that testing occurred in hypobaric hypoxia compared to normobaric hypoxia. The

physiological responses are different between altering the FiO_2 (hypoxia) for relatively short time intervals and altering the barometric pressure (hypobaric hypoxia) [12,16], thus our data should be considered novel and not necessarily generalizable to normobaric hypoxic findings. While our results show no ergogenic benefit of nitrate supplementation on forearm blood flow or grip endurance during the relative brief sojourn to altitude, this does not discount the possibility that nitrate supplementation may have positive benefits after a more prolonged exposure to high altitude where NO bioavailability has been found to be impaired [12].

4.3. Conclusion

The current study explored the effects of acute nitrate supplementation on forearm blood flow, tissue oxygenation and exercise performance during continuous and repeated rhythmic isometric grip exercise completed until task failure in hypobaric hypoxia at 4300 m and normobaric normoxia. Our results demonstrated impaired oxygen transport, lower forearm StO_2 and reduced exercise capacity during forearm grip exercise at altitude compared to exercise performed in normobaric normoxia. Despite increasing NO availability and significantly decreasing MAP during the CGrip exercise, acute nitrate supplementation did not significantly affect forearm blood flow, SaO_2 and StO_2 at rest and during grip exercise either at sea level pressure or during acute exposure to hypobaric hypoxia at 4300 m. In conclusion, these results indicate that acute nitrate supplementation has no ergogenic benefit during an initial exposure to high altitude. However, the possibility that nitrate supplementation may augment oxygen transport and exercise performance with longer altitude exposures, where NO bioavailability may be limited, should not be discounted and warrants further study.

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Conflict of interest

None.

Authorship

D.M.F. conception and design of research; H.G.G., A.R.R., A.R.L., S.E.S., and D.M.F. performed experiments; H.G.G., A.R.R., A.R.L., and D.M.F. analyzed data; D.M.F., H.G.G., and S.E.S. interpreted results of experiments; H.G.G. and D.M.F. drafted manuscript; H.G.G., S.E.S., and D.M.F. edited and revised manuscript; H.G.G., A.R.R., A.R.L., S.E.S., and D.M.F. approved final version of manuscript.

Disclosure

The views expressed in this article are those of the author(s) and do not necessarily reflect the official policy or position of the Uniformed Services University of the Health Sciences, Department of the Navy, Department of Defense, or the United States Government. “I am a military service member (or employee of the U.S. Government). This work was prepared as part of my official duties. Title 17, USC, §105 provides that ‘Copyright protection under this title is not available for any work of the U.S. Government.’ Title 17, USC, §101 defines a U.S. Government work as a work prepared by a military service member or employee of the U.S. Government as part of that person's official duties.”

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