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Analytical methods

# Effect of acute nitrate ingestion on central hemodynamic load in hypoxia☆,☆☆

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ABSTRACT

Acute hypoxia results in local vasodilation that may temporarily unload the left ventricle (LV) through nitric oxide (NO)-mediated mechanisms. Whether increasing NO levels augments LV unloading and improves ventricular-vascular coupling in hypoxia remains unknown.

*Purpose:* Investigate the effect of acute nitrate ingestion on central hemodynamic load in hypoxia.

*Methods:* 20 Healthy men (23 ± 3 yrs, BMI 24.6 ± 2.8 kg $m^{-2}$) consumed 70 mL of either a) 0.45 g nitrate (NIT) or b) an inert placebo (PLA) prior to 105 min of normobaric hypoxia (11.6 ± 0.1%) in this randomized, double-blind, crossover-design study. Wave reflection index (RIX; ratio of forward to reflected wave pressure), augmentation index (AIX75) and pulse wave velocity were calculated as measures of wave reflection magnitude and aortic stiffness, respectively, from the aortic blood pressure (BP) waveform. LV wasted pressure effort (WPE) was calculated as an index of LV work due to wave reflections. Subendocardial viability ratio (SEVR) was assessed a measure of myocardial $O_2$ supply/demand ratio.

*Results:* Aortic diastolic BP decreased in hypoxia compared to normoxia ($p < 0.05$). Aortic RIX, AIX75, and LV WPE significantly decreased in hypoxia compared to normoxia ($p < 0.05$). Aortic systolic BP, SEVR, and PWV were unaffected by hypoxia ($p > 0.05$). Compared to placebo, nitrate ingestion did not significantly alter central hemodynamics in hypoxia ($p > 0.05$).

*Conclusions:* Acute hypoxic exposure unloads the LV (WPE, AIX75, and RIX) without disturbing myocardial $O_2$ supply-demand ratio (SEVR). Reductions in LV work with hypoxia are likely due to reductions in pressure from wave reflections as hypoxia had negligible effects on aortic stiffness. Nitrate ingestion did not affect the central hemodynamic response to acute systemic hypoxia.



## 1. Introduction

The cardiovascular response to hypoxia is complex and consists of a balance between local vasodilation [1] and sympathetically mediated constriction [2]. Although it has been suggested that sympatholysis preserves most peripheral vasodilation in the face of hypoxia-induced sympathoexcitation [2], there may still be a net increase in arterial pressure [3–5] coupled with increases in heart rate. This combined hemodynamic effect can increase afterload and thus cardiac work. Indeed, acute hypoxic exposure often results in augmented cardiac work, driven by increases in cardiac output via chronotropic and inotropic factors [6,7]. Moreover, increased cardiac work can be accompanied by signs of left ventricular (LV) systolic dysfunction in hypoxic environments [8]. These cardiac responses to hypoxia can precipitate myocardial oxygen supply-demand imbalance and ischemia in some individuals [9], making this physiological response of particular concern to populations regularly exposed to environmental (i.e. mountaineers, military) and pathophysiological (i.e. clinical populations that suffer acute ischemic events) hypoxia. Ultimately, the effect of systemic hypoxia on cardiac work and myocardial energetics is poorly understood.

Aortic stiffness and pressure from wave reflections are important vascular-hemodynamic factors that influence LV work and myocardial energetics. Altered timing and magnitude of pressure from wave reflections affects afterload and LV work while also affecting coronary perfusion [10,11]. Recent studies note reductions in global wave reflection magnitude (assessed as the augmentation

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index) during hypoxia [4,5] which may have a favorable effect on unloading the LV and improving myocardial energetics in this setting. Reductions in global wave reflection magnitude are likely related to peripheral vasodilation and are partially nitric oxide (NO)-mediated, as NO-blockade increases augmentation index in hypoxia [5]. Nitric oxide (specifically nitrite reduction to NO) is an important modulator of hypoxic vasodilation [12,13]. Whether further increasing NO levels accentuates LV unloading in acute hypoxic conditions and improves myocardial energetics remains unexplored.

Nitric oxide can be synthesized endogenously by NO synthases using L-arginine and molecular oxygen [14] or a synthase-independent pathway utilizing nitrite [15]. This alternative pathway, referred to as the nitrate-nitrite NO pathway (for review see Ref. [16]), relies on NO's more-stable oxidation products of nitrate and nitrite, which act as a storage pool of NO substrate that can be readily converted to NO [17,18]. Generation of NO from nitrite was first identified in the stomach [19] but was later found to occur systemically [20] and thus have systemic vasoactive effects [13]. Nitrate is a major substrate and source of circulating nitrite [17] that is influenced by dietary intake (particularly vegetables [21]). Indeed, inorganic nitrate ingestion has been reported to have favorable effects on blood pressure [22,23], aortic stiffness [24], blood flow [22] and unload the LV under normoxic conditions [25]. Importantly, the reliance on nitrite as a source of intravascular NO appears to increase when synthase (and oxygen) -dependent pathways are inhibited [26]. The reduction of nitrite to NO and the consequent vasodilatory effects are amplified under hypoxic conditions [12,13,18]. In this manner, acute nitrate ingestion during hypoxic exposure could increase the available nitrite pool and NO formation, thereby enhancing vasodilation and reducing central blood pressure and pressure from wave reflections. The vasoactive effects of inorganic nitrate in this setting could improve ventricular-vascular coupling and myocardial energetics which may be beneficial during the transient increase cardiac work during initial hypoxic exposure.

The purpose of this study was to investigate if acute ingestion of a nitrate supplement would alter central hemodynamic load in hypoxia. It was hypothesized that nitrate ingestion would reduce central hemodynamic load and improve ventricular-vascular coupling in acute hypoxia compared to an inert placebo manifesting as greater reductions in aortic stiffness, pressure from wave reflections, and LV pressure effort coupled with increases in the subendocardial viability ratio (a measure of myocardial energetic balance).

## 2. Methods

Twenty healthy, active, non-anemic men (23 ± 3 yrs, BMI 24.6 ± 2.8 kg $m^{-2}$) participated in this study as a part of a larger investigation on the effects of nitrate ingestion on cognitive function in hypoxia which is published elsewhere [27]. Select results are presented in both papers out of necessity (salivary nitrite, arterial oxygen saturation, brachial blood pressure). Participants were not taking any medications/supplements during their enrollment in the study and were non-smokers, free of hypertension, diabetes mellitus, hyperlipidemia, pulmonary disease, renal disease, neurological disease, or peripheral artery disease, as assessed by a self-reported questionnaire. Participants completed two experimental trials separated by ≥ 72 h, with testing conducted at the same time of day within participants. Participants arrived ≥3 h fasted and were instructed to avoid vigorous exercise, and consuming caffeine/alcohol the day of testing and abstain from high nitrate foods for 2 days prior to testing. All subjects signed a written informed consent approved by the Syracuse University Institutional Review Board.

Following normoxic-baseline measures, participants consumed 70 mL of either a) a 0.40–0.45 g nitrate bolus (Beet It Sports Shot; NIT) or b) an inert placebo (PLA) prior to hypoxic exposure in this randomized, double-blind, crossover-design study. Although both supplements (Beet It, James White Ltd, Ipswich) had identical appearance, taste, and caloric value, the placebo underwent a manufacturing process to deplete it of nitrate (placebo ≈ 0.01 mmol $NO_3^-$ vs nitrate-rich ≈6.5–7.0 mmol $NO_3^-$, concentrations reported by manufacturer). Similar doses of nitrate (≈5 mmol) have previously been shown to elicit significant increases in plasma nitrite concentration in hypoxia (15% $O_2$) [28].

Participants rested in the supine position for 10 min upon arrival before undergoing normoxic-baseline vascular testing and salivary nitrite assessment. Normoxic-baseline always occurred prior to experimental manipulation (NIT vs PLA) in order to serve as a control and ensure there were no day-to-day variations in measures at baseline. Participants then ingested either a) NIT or b) PLA just prior to entering a normobaric hypoxic chamber ($FiO_2$ 11.6 ± 0.1%, ≈4,600 m; Hypoxico Systems, New York, NY). Participants remained in hypoxia for 1 h and 45 min before undergoing hypoxic-vascular testing. Our timeline was designed such that vascular-hemodynamic testing would occur concordant with peak nitrite availability (≈2 h post-nitrate ingestion) as suggested by previous literature [29]. All vascular measures were assessed in the supine position. Arterial oxygen saturation was measured using pulse oximetry, with a probe secured on the forehead (Nonin Medical, Plymouth, MN).

### 2.1. Salivary nitrite

Salivary nitrite was semi-qualitatively assessed using salivary test strips (Berkeley Test, Berkeley, CA) at normoxic-baseline (pre-ingestion) and immediately following vascular measures in hypoxia (≈2 h post-ingestion). Absorbed saliva was pressed against a reagent pad and after undergoing a chemical reaction, the reagent pad turn a color ranging from dull pink to dark pink. The resulting color was compared to a visual color scale that included 5 different categorical colors which were assigned a number from 0 to 4. According to the manufacturer, the 5 categories (0–4) correspond to approximate salivary nitrite concentrations of 21, 108, 217, 434, and 869 uM, respectively. We present the salivary nitrite concentrations in arbitrary units based on the 0–4 scale due to the limited fidelity in the test strips.

### 2.2. Brachial blood pressure

Systolic (SBP) and diastolic brachial blood pressure (DBP) were measured in duplicate prior to each set of vascular measures (at normoxia and hypoxia) using a validated, automated oscillometric cuff (EW3109, Panasonic Electric Works, Secaucus NJ). Mean arterial pressure (MAP) and pulse pressure (PP) were calculated as 1/3 systolic pressure + 2/3 diastolic pressure, and systolic pressure − diastolic pressure, respectively.

### 2.3. Aortic blood pressure

Radial pressure waveforms were collected in duplicate and averaged using applanation tonometry (SphygmoCor, AtCor Medical, Syndey, Australia). Waveforms were algorithmically transformed using a validated [30–32] generalized transfer function to estimate an aortic pressure waveform which was calibrated to brachial systolic and diastolic pressure. Additional variables derived from the synthesized aortic pressure wave included augmentation index (AIX), systolic pressure-time integral (SPTI), diastolic