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Title: UBC-Nepal Expedition: The use of oral antioxidants does not alter cerebrovascular function at sea-level or high-altitude

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Author Conflict: No competing interests declared

Running Title: Cerebrovascular function with antioxidants at 344 m and 5050 m

Abstract: There is evidence, albeit limited, that elevations in oxidative stress lead to impairments in cerebrovascular function in humans. The administration of antioxidants attenuates oxidative stress and may provide a mechanism of improving vascular function in situations where free radical production is excessive. One such scenario is exposure to hypoxia, where free radicals are elevated and vascular function is impaired; however, it remains to be determined if excessive oxidative stress is a relevant

mechanism underpinning hypoxia-mediated impairments of cerebrovascular function. Thus, the purpose of the current study was to examine the influence of orally ingested antioxidants at clinically relevant doses (vitamin C, E, and alpha-lipoic acid) on cerebrovascular regulation at sea-level (344 m; n=12 participants) and at high altitude (5050 m; n=9) in a randomized, placebo-controlled and double-blinded crossover design. Hypercapnic and hypoxic cerebrovascular reactivity tests [internal carotid (ICA) and middle cerebral artery] were conducted at sea level while global and regional cerebral blood flow [ICA and vertebral artery flow (VA)] were assessed after 10-12 days following arrival at 5050 m. At sea level, acute administration of antioxidants did not alter cerebral vascular reactivity (hypoxic; pre vs post [1.50.7 vs 1.20.8 %ΔCBF/-%ΔSpO2; P=0.96] or hypercapnic; pre vs post [5.72.0 vs 5.81.9 %ΔCBF/ΔmmHg; P=0.33]). Furthermore, global cerebral blood flow (P=0.43) as well as cerebral vascular conductance (ICA CVC P=0.08; VA CVC P=0.32) were unaltered at 5050 m following antioxidant administration. In conclusion, oral administration of antioxidants does not influence cerebrovascular function or blood flow at sea-level or high altitude.

New Findings: What is the central question of the study? Does the use of antioxidants alter cerebrovascular function and blood flow at sea-level (344 m) and/or high-altitude (5050 m)? What is the main finding and importance? This is the first study to investigate whether antioxidant administration alters cerebrovascular regulation and blood flow in response to hypercapnia, acute hypoxia and chronic hypoxia in healthy humans. We demonstrate that an acute dose of antioxidants does not alter cerebrovascular function and blood flow at sea-level (344 m) or following 12 days at high-altitude (5050 m).

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UBC-Nepal Expedition: The use of oral antioxidants does not alter cerebrovascular function at sea-level or high-altitude

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New Findings

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What is the main finding and importance?

This is the first study to investigate whether antioxidant administration alters cerebrovascular regulation and blood flow in response to hypercapnia, acute hypoxia and chronic hypoxia in healthy humans. We demonstrate that an acute dose of antioxidants does not alter cerebrovascular function and blood flow at sea-level (344 m) or following 12 days at high-altitude (5050 m).

Abstract

There is evidence, albeit limited, that elevations in oxidative stress lead to impairments in cerebrovascular function in humans. The administration of antioxidants attenuates oxidative stress and may provide a mechanism of improving vascular function in situations where free radical production is excessive. One such scenario is exposure to hypoxia, where free radicals are elevated and vascular function is impaired; however, it remains to be determined if excessive oxidative stress is a relevant mechanism underpinning hypoxia-mediated impairments of cerebrovascular function. Thus, the purpose of the current study was to examine the influence of orally ingested antioxidants at clinically relevant doses (vitamin C, E, and alpha-lipoic acid) on cerebrovascular regulation at sea-level (344 m; n=12 participants) and at high altitude (5050 m; n=9) in a randomized, placebo-controlled and double-blinded crossover design. Hypercapnic and hypoxic cerebrovascular reactivity tests [internal carotid (ICA) and middle cerebral artery] were conducted at sea level while global and regional cerebral blood flow [ICA and vertebral artery flow (VA)] were assessed after 10-12 days following arrival at 5050 m. At sea level, acute administration of antioxidants did not alter cerebral vascular reactivity (hypoxic; pre vs post [1.5±0.7 vs 1.2±0.8 %ΔCBF/-%ΔSpO_2; P=0.96] or hypercapnic; pre vs post [5.7±2.0 vs 5.8±1.9 %ΔCBF/ΔmmHg; P=0.33]). Furthermore, global cerebral blood flow (P=0.43) as well as cerebral vascular conductance (ICA CVC P=0.08; VA CVC P=0.32) were unaltered at 5050 m following antioxidant administration. In conclusion, oral administration of antioxidants does not influence cerebrovascular function or blood flow at sea-level or high altitude.

Words: 247

Introduction

Oxidative stress refers to the imbalance between the production of pro-oxidants (e.g. free radicals) and available antioxidants (e.g. vitamin C). The physiological effect of oxidative stress is determined by the magnitude of this imbalance; homeostasis consists of low levels of oxidative stress (Trinity et al., 2016) while high-levels – albeit as of yet not precisely defined concentrations – are pathological and have been implicated in the structural damage and vascular dysfunction observed in multiple disease states (Ives et al., 2014; Lavi et al., 2006; Rossman et al., 2015; Ting et al., 1996).

Elevated markers of oxidative stress are evident in both acute (Irarrázaval et al., 2017) and chronic hypoxia (Lewis et al., 2014), which may lead to a reduction in nitric oxide (NO) bioavailability through the interaction of NO and superoxide (i.e. free radical [O_2^-]) to form peryoxynitrite ($O_2^- + NO = ONOO^-$) (Meli et al., 2002; Thomas et al., 2008). This reduction in NO bioavailability may ultimately decrease peripheral vascular function (Green et al., 2011) as well as cerebrovascular CO_2 (Smith et al., 1997) and hypoxic (Bailey et al., 2009) reactivity. Thus, as administration of antioxidants reduces overall total oxidative stress concentrations (Richardson et al., 2007) this may improve cerebrovascular function. Excessive increases in oxidative stress have been observed in clinical populations with impaired cerebrovascular reactivity to CO_2 such as diabetes (Lavi et al., 2006; Ting et al., 1996) and acute ischemic stroke (Kotur-Stevuljevic et al., 2015; Reinhard et al., 2004).

While animal studies have demonstrated no effect of elevated oxidative stress on cerebrovascular CO_2 reactivity (e.g., experimental increases in the hydroxyl radical) (Leffler et al., 1991; Rosenblum, 1983) the limited studies conducted to date in humans indicate oxidative stress may in fact impair cerebral reactivity (Hartmann et al., 2015; Hartmann et al., 2012).

Nevertheless, the paucity of data in humans (especially healthy humans) is quite surprising given the susceptibility of the brain to changes in the redox balance. Further, studies conducted to date have been methodologically limited as only data on intra-cranial cerebral blood velocity responses to antioxidants using transcranial Doppler (TCD) ultrasound have been collected. For example, in 2015, Hartmann and colleagues reported that intravenous infusion of vitamin C (three grams) reduced cerebral blood velocity during acute hypoxia in young healthy individuals. There are, however, methodological issues associated with TCD as the technique assumes the diameter of the insonated intra-cranial vessel (e.g. middle cerebral artery, [MCA]) remains constant in hypoxia (Hartmann et al., 2015). Given the recent criticisms of this assumption [reviewed in: (Hoiland & Ainslie, 2017)], further assessment of the role of antioxidants in cerebrovascular regulation is warranted where both diameter and velocity are both assessed.

Using a previously validated antioxidant cocktail (Richardson et al., 2007; Wray et al., 2012) combined with high resolution duplex ultrasonography, in two separate studies, we investigated if oral administration of antioxidants alters cerebral hemodynamics: (1) during acute exposure to hypercapnia and normobaric hypoxia, and (2) following a 9-day trekking ascent and subsequent12-days at high-altitude (5050 m). Both studies employed a double blinded, randomized, placebo controlled design at sea level (Study 1) and high altitude (Study 2). In Study 1, we hypothesized that the cerebral vascular response to hypercapnia and hypoxia would be reduced after the administration of oral antioxidants in young healthy subjects at sea-level. In Study 2 we anticipated that global cerebral blood flow (gCBF) would be increased following administration of oral antioxidants in young healthy subjects at high-altitude.

Methods

Ethical Approval

All experimental procedures were approved by University of British Columbia Clinical Research Ethics Board (CREB ID: H16-00101) and the Nepal Health Research Council, and conformed to the standards set by the *Declaration of Helsinki*. Verbal and written consent were obtained from all participants.

Study 1 – Sea-level testing (344 m):

Participants

Twelve healthy young individuals (age: 24±3 years of age; two females; body mass index 24±2kg/m^2) volunteered to participate in Study 1. Participants were screened to ensure reliable ultrasound measurements of the internal carotid artery (ICA). All participants were free of cardiovascular, respiratory & cerebrovascular diseases, were non-diabetic, and were not taking any prescription drugs (other than oral contraceptives n=2) at the time of participation. A dietary food restriction sheet was given to all participants so that they could avoid foods high in antioxidants and nitrates (Kapil et al., 2014).

Protocol design

Figure one illustrates the protocol design for studies 1 & 2. Study 1 required two separate laboratory visits that were separated by at least 48 hrs to account for adequate antioxidant washout (Bailey & Davies, 2001; Richardson et al., 2007). Participants arrived at the laboratory having abstained from caffeine for 12 hours, exercise and alcohol for 24 hours, and had fasted for 2 hours following a light meal. Upon arrival, participants were asked to lie in the supine position for 15-minutes while they were instrumented (see 'Experimental Measures' below).

After the 15-minute rest period, baseline ventilatory, cardiovascular, and cerebrovascular data were recorded during five minutes of room air breathing. Participants then breathed on an

end-tidal forcing system (see 'Experimental Measures' below) where their room air partial pressures of end-tidal carbon dioxide and oxygen ($P_{ET}O_2$ and $P_{ET}CO_2$) (e.g. $P_{ET}O_2$ = 91.1 ± 13.0 mmHg & $P_{ET}CO_2$ = 42.4 ± 2.3 mmHg) were held constant for two-minutes. Following the two-minute room air baseline period, the participants underwent five minutes of iso-oxic hypercapnia (+9 mmHg $P_{ET}CO_2$). Cerebral blood flow (CBF) and cerebral blood velocity of the ICA and MCA, respectively, were recorded during baseline and the last minute of hypercapnia. A five-minute recovery period followed the hypercapnia stage, before a second baseline was recorded for two-minutes followed by 10-minutes of isocapnic hypoxia ($P_{ET}O_2$ = 45mmHg) with the $P_{ET}O_2$ targeted to mimic the level of $P_{ET}O_2$ at 5000 m based on a previous investigation by our group (Lewis et al., 2014). Ultrasound images were recorded throughout the two-minute baseline and the ten-minute trial, with the final minute used for analysis. After all measurements and recordings were completed, the participant ingested either a placebo (appearance and weight matched sugar pills) or antioxidant dose of vitamin C (500 mg), vitamin E (400 IU), and alpha lipoic acid (300 mg). A second dose (antioxidant or placebo) containing vitamin C (500 mg), vitamin E (200 IU), and alpha lipoic acid (300 mg) was ingested approximately 30-minutes after the first antioxidant dose. The use of this specific oral antioxidant dose has been previously established showing increased antioxidant levels within the blood and decreased systemic free radical-mediated lipid peroxidation (Richardson et al., 2007; Wray et al., 2012). Post-drug/placebo experimentation resumed exactly 60-minutes after the second dose of either placebo or antioxidants (Bailey & Davies, 2001; Richardson et al., 2007).

198 **Study 2 - High altitude testing (5050 m):**

199 **Participants**

200 Nine healthy young individuals (age: 24±4yrs; two females; body mass index:
201 23±2kg/m^2) volunteered for Study 2. All participants were born and lived close to sea-level
202 (<1500 m). Participants were screened to ensure reliable ultrasound measurements of the ICA.
203 All participants were free of cardiovascular, respiratory & cerebrovascular diseases, were non-
204 diabetic, and were not taking any prescription drugs or antioxidant supplements (other than oral
205 contraceptives n=1, and IUD n=1) at the time of or prior to participation.

206 **High-altitude protocol design (Figure 1)**

207 Similar to Study 1, the design was double blinded, randomized and placebo controlled.
208 Each laboratory visit was separated by 48 hrs to ensure adequate antioxidant washout
209 (Richardson et al., 2007). Each trial was completed within the Ev-K2-CNR Pyramid laboratory
210 located in Khumbu Valley, Nepal (5050 m), and this study was a part of a larger research
211 expedition conducted between September and November of 2016 which is currently under
212 review. In brief, ascent to the Pyramid Laboratory took place over a slow and safe 9-day trekking
213 protocol without the use of any acute mountain sickness prophylactics (e.g., acetazolamide).
214 Participants spent one night in Monjo (2800 m), three nights in Namche Bazaar (3400m), one
215 night in Deboche (3820 m), and then three nights in Pheriche (4371 m) followed by the final
216 trekking day to the Pyramid laboratory (5050 m), and 12 days after initial arrival. While at the
217 Pyramid laboratory, participants took part in several studies that were conducted throughout the
218 three-week period, but careful consideration of washout times and recovery meant there was no

crossover between studies. Moreover, the *a priori*, primary research questions addressed in the current paper are novel and are exclusively dealt with in this study alone.

Participants were examined after refraining from caffeine for 12 hours, exercise and alcohol for 24 hours, and were fasted for at least two hours. Participants were instrumented and cardiorespiratory measures were taken at the start of both the pre- and post-testing trials. Participants were requested to lie quietly in the supine position for 15-minutes upon arrival. Following the collection of the cardiorespiratory variables detailed below, two-minute ultrasound recordings of both the ICA and vertebral artery (VA) were obtained. Immediately following these CBF measures, similar to the study 1, participants ingested two doses of either placebo or antioxidants (vitamin C, E, alpha-lipoic acid).

Experimental measures

Study 1- sea-level

All cardiorespiratory variables were sampled continuously throughout the protocol at 1KHz via an analogue-to-digital converter (Powerlab, 16/30; ADInstruments, Colorado Springs, CO, USA). Heart rate (HR) was measured by a 3-lead electrocardiogram (ADI bioamp ML132; ADInstruments, Colorado Springs, CO, USA), and beat-to-beat blood pressure by finger photoplethysmography (Finometer PRO, Finapres Medical Systems, Amsterdam, Netherlands). The Finometer reconstructed brachial waveform was used for the calculation of mean arterial pressure (MAP) after values were back calibrated to the average of three automated brachial blood pressure measurements made over 5-minutes at rest (Tango+; SunTech, Morrisville, NC, USA). Participants' $P_{ET}CO_2$, and $P_{ET}O_2$ were sampled at the mouth, and recorded by a calibrated gas analyzer (model ML206; ADInstruments, Colorado Springs, CO, USA). Ventilation (V_E), tidal volume, and breathing frequency were measured by a pneumotachograph (model HR 800L,

HansRudolph, Shawnee, KS, USA) connected in series to a bacteriological filter. Peripheral oxyhemoglobin saturation (SpO_2), was measured using a pulse-oximeter. All data measurements were displayed on LabChart (version 7.1; ADInstruments, Colorado Springs, CO, USA), and analyzed offline. Analysis and collection of all data were performed blinded to the condition (i.e., placebo vs antioxidants).

Blood velocity and vessel diameter of the ICA were measured using a 10MHz multi-frequency duplex ultrasound (Terason T3200, Teratech, Burlington, MA, USA). Arterial diameter was assessed with B-mode imaging while pulse-wave mode was used to simultaneously measure peak blood velocity. Measures of ICA blood flow (Q_{ICA}) were made ipsilateral to MCA. Blood vessel diameter and velocity of ICA were measured at least 1.5 cm distal from the common carotid bifurcation to eliminate measures of turbulent and retrograde flow. Blood velocity through the right MCA (MCAv) was measured using a 2MHz transcranial Doppler ultrasound (TCD; Spencer Technologies, Seattle, WA, USA). The TCD probes were secured in place using a specialized headband (model M600 bilateral head frame, Spencer technologies, Redmond, WA, USA) using standardized techniques (described in Willie et al., 2011).

In Study 1, both $P_{ET}CO_2$ and $P_{ET}O_2$ were controlled using a dynamic end-tidal forcing system. The system uses independent gas solenoid valves for O_2, CO_2, and nitrogen (N_2) and controls the volume of each gas delivered into a reservoir through a mixing and humidification chamber. The $P_{ET}O_2$, $P_{ET}CO_2$, expiratory and inspiratory tidal volume, frequency of breathing, and minute ventilation were determined for each participant on a breath-by-breath basis in real time using custom designed software (Labview 13.0, National Instruments, Austin, TX, USA). The operation and use of our dynamic end-tidal forcing system has been previously described in detail (Tymko et al., 2016; Tymko et al, 2015). Cerebral reactivity to hypercapnia was calculated

using the baseline cerebral blood flow measure subtracting the final minute of cerebral blood flow measures during hypercapnia, then dividing by the overall change in $P_{ET}CO_2$. Similarly, the following formula was used for the calculation of hypoxic reactivity = $\%\Delta CBF/\%\Delta SpO_2$.

Study 2 – high-altitude

Systolic, diastolic and MAP were recorded along with HR from an automated blood pressure cuff (HEM-775CAN, Omron Healthcare, Bannockburn, IL, USA). Respiratory rate and $P_{ET}CO_2$ were measured from a portable device (EMMA, Mainstream Capnometer, Danderyd, Sweden), and SpO_2 from a finger pulse oximeter (Vacu-med, Ventura, California, USA).

In Study 2, ICA flow was assessed as described above. In addition, the diameter and velocity of the vertebral artery (VA) were also measured between C4-C5, C5-C6, or proximal to entry into the vertebral column. These locations were determined on an individual basis, with the same location repeated in each participant to acquire reproducible measures. The use of TCD to assess MCAv was not used in the high-altitude protocol.

At high-altitude and sea-level volumetric blood flow was calculated using the following formula:

$$Q_{ICA}, \text{ or } Q_{VA} = \frac{\text{Peak Envelope Velocity}}{2} * [\pi(0.5*\text{Diameter})^2]$$

For the high-altitude study, gCBF was calculated using the following formula:

$$gCBF = (Q_{ICA} + Q_{VA}) \cdot 2$$

Cerebrovascular conductance (CVC) was determined to account for differences in MAP during analysis of CBF responses. This was calculated for both ICA and VA (e.g., Q_{ICA}/MAP and Q_{VA}/MAP). Screen capture software was used for ultrasound recordings, and video files were stored for offline analysis. Analysis videos of both the ICA and VA were recorded and analyzed while blinded to the protocol (placebo vs antioxidant). Edge detection and wall-

tracking software were used to determine measures of vessel diameter and peak blood velocity, at least twelve consecutive cardiac cycle were used to determine Q_{ICA} and Q_{VA} (Woodman et al., 2001).

Data Analysis

Statistical analysis for both sea-level and high-altitude were performed using IBM SPSS (Version 24, IBM statistics), and are reported as mean ± standard deviation (SD). Statistical significance was assumed at $P < 0.05$. Data analysis was separated via the two different studies. For study 1, a two-way (Study 1: drug/placebo x time) repeated measures analysis of variance (ANVOA) were used to compare CBF and cardiovascular variables either pre/post antioxidant or placebo for both the hypoxia and hypercapnic trials. For study 2, a two-way (Study 2: drug x time) repeated measures ANOVA was used to compare CBF and cardiovascular variables with the antioxidant and placebo trial as a function of pre versus post intervention. For both studies, when interaction effects were detected pairwise comparisons were made using Bonferroni-corrected t-tests.

Results

Study 1- Acute hypercapnia and hypoxia

Cardiovascular and cerebrovascular baseline variables for placebo and antioxidant with iso-oxic hypercapnia (+9 mmHg $P_{ET}CO_2$) and isocapnic hypoxia (45 mmHg $P_{ET}O_2$) are presented in Table 1 and 2, respectively. Both hypercapnia and hypoxia elevated MAP, HR, and V_E ($P<0.05$ for both trials). During the hypercapnic trials, $P_{ET}O_2$ was maintained at each participant baseline levels ($P_{ET}O_2$ = 92.5 vs 93.2 mmHg; P=0.77) for each testing trial. Similarly, during the hypoxic trials $P_{ET}CO_2$ was maintained at each participant baseline levels ($P_{ET}CO_2$ =

43.0 vs 42.7 mmHg; P=0.67) for each testing trial. There were no significant differences in the magnitude of end-tidal gas manipulations between placebo and antioxidant. Hypercapnia increased Q_{ICA} (P<0.001), ICAv (P=0.01), ICA diameter (P=0.001), ICA CVC (P<0.001), and MCAv (P<0.001). Similarly, Q_{ICA} (P=0.01), ICAv (P=0.01), ICA diameter (P=0.001), ICA CVC (P=0.03), and MCAv (P=0.001) all increased during isocapnic hypoxia. There were no between-trial (i.e., placebo vs. antioxidant) differences for any cerebrovascular variables. Figure 2 shows individual data of both hypoxic and hypercapnic reactivity within the ICA pre and post in placebo and antioxidants.

Study 2- Chronic Hypoxia

Cardiovascular and cerebral vascular variables are presented in Table 4. There were no significant differences in MAP or SpO_2 pre-versus post administration of either antioxidants or placebo. Similarly, there was no significant changes in Q_{ICA} (P=0.22), ICAv (P=0.83), ICA diameter (P=0.19), ICA CVC (P=0.07), Q_{VA} (P=0.26), VAv (P=0.20), VA diameter (P=0.11), and VA CVC (P=0.32). Figure 3 demonstrates that there were no significant alterations in gCBF following placebo or antioxidant treatment (P=0.43).

Discussion

This is the first study to investigate whether antioxidant administration alters cerebrovascular regulation and blood flow in response to hypercapnia, acute hypoxia, and chronic hypoxia in healthy humans. The primary findings from our study were that the administration of oral antioxidants did not alter (i) hypercapnic or hypoxic cerebrovascular reactivity at sea-level (344 m) or (ii) gCBF at high-altitude (5050 m). Collectively, these findings highlight that at both sea-level and high-altitude, acute antioxidant administration does not alter cerebral vascular function in young healthy humans.

331

332 *Cerebrovascular function, reactive oxygen species and antioxidants*

333 We found no significant change in either MCAv or ICA flow reactivity to hypoxia at
334 sea-level. A previous study performed by Hartmann et al., (2015) reported a decrease in MCAv
335 reactivity to isocapnic hypoxia in young healthy participants following intravenous infusion of
336 vitamin C (three grams) (Hartmann et al., 2015). Differences between the findings of Hartmann
337 et al. (2015) and the present study may be explained by their use of TCD to obtain velocity and
338 not flow, as recent evidence has suggested that the MCA dilates in hypoxia (Verbree et al.,
339 2014), and that just using MCAv would ultimately underestimate cerebral blood flow (Hoiland &
340 Ainslie, 2017). In the current study, we observed dilation within the ICA during both
341 hypercapnia and acute hypoxia trials. Although, the physical properties (i.e. anatomical size and
342 compliance) between intracranial and extracranial compartments differ, these data alone are
343 consistent with previous reports of dilation of the ICA as well as the MCA during changes in
344 arterial blood gases (Hoiland, et al., 2017; Imray et al., 2014; Wilson et al., 2011). Another
345 consideration is that Hartmann and colleagues (2015) utilized a supra-physiological intravenous
346 dose of vitamin C (three grams vs. the upper recommended limit for daily intake of two grams).
347 This dose was speculated to have a pro-oxidant effect, instead of antioxidant effect (Hartmann et
348 al., 2015). The dosing strategy used within the current study is likely more physiologically
349 relevant. Further, the use of only a water-soluble antioxidant (i.e. vitamin C), as was utilized by
350 Hartmann and colleagues (2015), reduces aqueous superoxide and alkoxyl radicals (Bailey &
351 Davies, 2001), but has a reduced ability to scavenge peroxyl radicals and related chain-breaking
352 abilities (Regoli & Winston, 1999). In contrast, lipid-soluble antioxidants target peroxyl radicals
353 (Burton & Ingold, 1989). Therefore, our current study used an antioxidant cocktail that contained

water (e.g. vitamin C) and lipid (e.g. α-tocopherol) soluble chain-breaking antioxidants in combination with α-lipoic acid, a unique "ideal" antioxidant that is both water and lipid soluble (Packer et al., 1997). This dosing combination allows for greater oxidative stress scavenging, as well as previously stated this dosing has been proven to reduce oxidative stress levels within humans (Richardson et al., 2007).

Similar to the hypoxia data, we found no significant change in either MCAv or ICA flow reactivity to hypercapnia at sea-level after the administration of oral antioxidants. However, there may be an equal or greater influence of other redundant and compensatory mechanisms influencing vasomotor tone within the cerebral arteries during increased alterations of arterial CO_2 other than strictly NO-mediated signal transduction (Smith et al., 1997) such as prostaglandins which up-regulate cAMP causing vasodilation (Hoiland et al., 2016; Pelligrino & Wang, 1998; St Lawrence et al., 2002), cerebral autoregulation (Ogoh et al., 2010), and sympathetic activity (Peebles et al., 2012). In relation, in COPD patients, it was demonstrated using statistical covariate analysis that the elevated oxidative stress characteristic of COPD patients may underpin their impaired CO_2 reactivity when compared to controls (Hartmann et al., 2012). However, this study only assessed cerebral blood velocity via TCD. Thus, the lack of experimental manipulation of oxidative stress and limited technique for CBF measurement make it difficult to draw strong inferences.

High altitude, cerebrovascular function, and antioxidants

The novelty of this current study was that it is the first to assess gCBF after acclimatization to altitude with and without the use of antioxidants. There are well reported elevations in oxidative stress with normobaric (Irarrázaval et al., 2017) and hypobaric hypoxia (Bailey et al., 2013; Lewis et al., 2014). Therefore, it is reasonable to hypothesize that this

increase in oxidative stress may, in part, be responsible for the alternations in cerebral vascular function at high altitude (Jensen et al., 1996). Within Study 2, we reported the same consistent gCBF values that were previously reported following ~ one week of acclimatization at the same altitude [e.g. 815 ± 50 ml/min (Willie et al., 2014)]. Resting CBF remained unchanged at altitude after the administration of acute oral antioxidants. As subjects were acclimatized and CBF had returned to sea-level values (650 ± 68 ml/min) the antioxidant intervention may have had no effect as CBF was already "normal". Whether or not chronic antioxidant supplementation throughout the overall ascent or an increased dose may have resulted in different results remains to be established.

Methodological considerations

Large quantities of peroxidizable polyunsaturated fatty-acid side chains and a limited antioxidant defense leave the brain highly susceptible to high levels of oxidative stress (Bailey et al., 2009). Furthermore, there are high concentrations of iron stores (McCord & Day, 1978) in which interactions with oxidative stress may potentially lead to lipid peroxidation (Gutteridge et al., 1983). Collectively, these mechanisms may leave the brain more susceptible to oxidative stress and consequent dysfunction. Thus, it is somewhat surprising antioxidants had no effect; however, this dosage has been demonstrated to reduce oxidative stress in peripheral venous blood samples which may not be fully reflective of the cerebral vascular environment (Richardson et al., 2007).

This current study was able to examine the effects of oral antioxidant supplementation on cerebrovascular regulation at both sea-level and high-altitude. As well, it implemented a double-blinded, placebo controlled experimental design to account for any between-day variability unlike previous studies. There are, however, a number of limitations related to our altitude study

we must acknowledge. First, with any high-altitude field study comes a number of potential confounders (e.g. altitude illness, increased sympathetic nervous activity, and subject physical conditions). Second, while the measures applied in Study 1 provide greater insight into cerebrovascular regulation, we were not able to replicate the design due to logistical issues associated with high altitude field research and only quantified resting gCBF at HA. Third, we were only able to recruit a relatively small sample size at altitude (n=9). Lastly, this study did not measure blood samples to track changes in oxidative stress both at sea-level and high-altitude; however, we chose an identical dosing strategy previously shown to increase antioxidant levels and reduce oxidative stress (Richardson et al., 2007).

Conclusion

In conclusion, oral administration of antioxidants does not influence cerebrovascular function and blood flow at sea-level or high altitude, respectively.

Author contributions

Conception and design of experiments: ABH, PNA. Data Collection: ABH, RLH, NSCL, MMT, JCT, HHC, DNF, MS, PNA. Data analysis and interpretation: ABH, RLH, MMT, MS, NSCL, PNA. Manuscript first draft: ABH, PNA. Critical revisions of manuscript for important intellectual content: ABH, RLH, MMT, NSCL, JCT, MS, DNF, HHC, DMB, PNA. Approval of final draft: ABH, RLH, NSCL, MMT, JCT, MS, DNF, HHC, DMB, PNA.

Disclosure

The authors disclose no conflicts of interests

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Tables:

Table 1. Cerebral vascular, hemodynamics, and respiratory variables at baseline and during iso-oxic hypercapnia following placebo or antioxidants. All data are presented as mean ± SD.

		Placebo		Antioxidant	
		Pre	**Post**	**Pre**	**Post**
$P_{ET}CO_2$ (mmHg)	**Baseline**	42.4 ± 2.3	42.3 ± 2.0	43.4 ± 2.1	42.9 ± 2.2
	CO_2	52.3 ± 2.4*	51.8 ± 1.8*	52.8 ± 1.8*	52.7 ± 2.2*
P value	Pre vs Post P=0.89; CO_2 P<0.05; Interaction P<0.05			Pre vs Post P=0.89; CO_2 P<0.05; Interaction P=0.58	
VE ($L \cdot min^{-1}$)	**Baseline**	13.1 ± 3.5	14.5 ± 5.3	13.8 ± 4.8	14.1 ± 4.5
	CO_2	40.0 ± 15.1*	37.6 ± 16.1*	38.2 ± 13.0*	39.9 ± 13.2*
P value	Pre vs Post P=0.84; CO_2 P<0.05; Interaction P=0.40			Pre vs Post P=0.36; CO_2 P<0.05; Interaction P=0.44	
MAP (mmHg)	**Baseline**	89.2 ± 11.8	88.6 ± 9.8	91.5 ± 8.1	91.7 ± 13.4
	CO_2	97.8 ± 14.0*	96.7 ± 9.4*∞	96.6 ± 6.3*	97.4 ± 8.7∞
P value	Pre vs Post P<0.05; CO_2 P<0.05; Interaction P<0.05			Pre vs Post P=0.64; CO_2 P<0.05; Interaction P=0.86	
HR ($beats \cdot min^{-1}$)	**Baseline**	63.7 ± 10.9	59.5 ± 10.4	59.9 ± 9.4	61.2 ± 11.9
	CO_2	74.0 ± 13.2	68.6 ± 16.0	68.0 ± 9.0*	68.1 ± 12.7*
P value	Pre vs Post P=0.09; CO_2 P=0.09; Interaction P=0.79			Pre vs Post P=0.70; CO_2 P<0.05; Interaction P=0.63	
SpO_2 (%)	**Baseline**	96.4 ± 2.8	97.7 ± 1.2	97.8 ± 0.7	96.4 ± 3.7
	CO_2	96.3 ± 2.8	97.3 ± 0.9	97.8 ± 0.7	96.9 ± 0.9
P value	Pre vs Post P=0.05; CO_2 P=0.98; Interaction P=0.85			Pre vs Post P=0.19; CO_2 P=0.67; Interaction P=0.48	
Q_{ICA} ($mL \cdot min^{-1}$)	**Baseline**	266.8 ± 61.2	257.6 ± 71.1	274.5 ± 57.2	269.1 ± 65.4
	CO_2	415.3 ± 86.3*	418.4 ± 93.1*	416.8 ± 88.7*	413.9 ± 87.8*
P value	Pre vs Post P=0.17; CO_2 P<0.05; Interaction P=0.25			Pre vs Post P=0.37; CO_2 P<0.05; Interaction P=0.22	
ICAv ($cm \cdot s^{-1}$)	**Baseline**	45.4 ± 14.8	40.7 ± 9.5	42.8 ± 8.2	42.2 ± 9.6
	CO_2	62.6 ± 10.7*	60.0 ± 13.7*	60.0 ± 11.8*	60.0 ± 10.7*
P value	Pre vs Post P=0.07; CO_2 P<0.05; Interaction P=0.64			Pre vs Post P=0.88; CO_2 P<0.05; Interaction P=0.81	
ICA diameter (mm)	**Baseline**	5.2 ± 0.54	5.2 ± 0.95	5.2 ± 0.56	5.0 ± 0.42
	CO_2	5.3 ± 0.46*	5.4 ± 0.49*	5.5 ± 0.53*	5.4 ± 0.51*
P value	Pre vs Post P=0.99; CO_2 P<0.05; Interaction P=0.08			Pre vs Post P=0.50; CO_2 P<0.05; Interaction P=0.13	
ICA CVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	**Baseline**	3.5 ± 1.6	3.0 ± 0.9	2.9 ± 0.8	2.9 ± 1.1
	CO_2	4.3 ± 0.9*	4.3 ± 1.2*	4.1 ± 1.1*	4.3 ± 1.2*
P value	Pre vs Post P=0.21; CO_2 P<0.05; Interaction P=0.19			Pre vs Post P=0.24; CO_2 P<0.05; Interaction P=0.47	
MCAv ($cm \cdot s^{-1}$)	**Baseline**	62.8 ± 10.2	61.5 ± 9.9	60.9 ± 7.7	59.8 ± 8.6
	CO_2	86.8 ± 19.0*	84.0 ± 14.3*	84.7 ± 14.3*	83.3 ± 14.1*
P value	Pre vs Post P=0.29; CO_2 P<0.05; Interaction P=0.67			Pre vs Post P=0.35; CO_2 P<0.05; Interaction P=0.82	

For both between-trial placebo or antioxidant. Significance for CO_2 as a function of group is indicated by * ($P < 0.05$), significant difference for pre vs post as a function of group is indicated by ∞ ($P < 0.05$). abbreviations. Note. - $P_{ET}CO_2$ = end-tidal partial pressure of carbn dioxide; VE = ventilation; MAP = mean arterial pressure; HR = heartrate; SpO_2 = peripheral capillary saturation of oxygen; Q_{ICA} = blood flow through the internal carotid artery; ICA_V = velocity of blood through the internal carotid artery; ICA CVC = internal carotid artery cutaneous vascular conductance; MCAv = blood velocity through the middle cerebral artery.

Table 2. Cerebral vascular, hemodynamic, and respiratory variables at baseline and during isocapnic hypoxia following placebo or antioxidants. All data are presented as mean ± SD.

		Placebo		Antioxidant	
		Pre	**Post**	**Pre**	**Post**
$P_{ET}O_2$ (mmHg)	**Baseline**	93.5 ± 5.5	94.9 ± 4.0	93.3 ± 6.5	93.7 ± 5.1
	Hypoxia (45mmHg)	50.1 ± 4.3*	50.4 ± 4.2*	56.3 ± 4.0*	50.4 ± 4.1*
P value		Pre vs Post P=0.22; O_2 P<0.05; Interaction P=0.41		Pre vs Post P=0.36; O_2 P<0.05; Interaction P=0.30	
VE ($L \cdot min^{-1}$)	**Baseline**	13.7 ± 3.6	13.6 ± 3.8	12.6 ± 3.8	15.7 ± 3.3∞
	Hypoxia (45mmHg)	28.1 ± 7.4*	26.9 ± 8.7*	24.4 ± 10.4*	28.8 ± 11.4*∞
P value		Pre vs Post P=0.51; O_2 P<0.05; Interaction P=0.53		Pre vs Post P<0.05; O_2 P<0.05; Interaction P=0.55	
MAP (mmHg)	**Baseline**	93.8 ± 11.4	93.8 ± 10.7	91.8 ± 9.6	92.3 ± 11.0
	Hypoxia (45mmHg)	98.1 ± 13.4*	99.3 ± 13.7*	95.6 ± 10.4*	97.3 ± 9.1*
P value		Pre vs Post P=0.85; O_2 P<0.05; Interaction P=0.50		Pre vs Post P=0.83; O_2 P<0.05; Interaction P<0.05	
HR ($beats \cdot min^{-1}$)	**Baseline**	60.2 ± 10.5	60.9 ± 11.7	59.5 ± 8.8	60.0 ± 8.9
	Hypoxia (45mmHg)	73.2 ± 16.8*	70.6 ± 17.6*	72.2 ± 14.7*	75.1 ±14.2*
P value		Pre vs Post P=0.60; O_2 P<0.05; Interaction P=0.13		Pre vs Post P=0.16; O_2 P<0.05; Interaction P=0.33	
SpO_2 (%)	**Baseline**	97.5 ± 1.2	97.8 ± 0.9	97.7 ± 0.9	97.9 ± 1.1
	Hypoxia (45mmHg)	82.7 ± 4.9*	82.4 ± 4.8*	82.7 ± 4.8*	82.5 ± 4.9*
P value		Pre vs Post P=0.94; O_2 P<0.05; Interaction P=0.72		Pre vs Post P=0.86; O_2 P<0.05; Interaction P=0.19	
Q_{ICA} ($mL \cdot min^{-1}$)	**Baseline**	224.8 ± 81.4	247.5 ± 78.8	244.9 ± 77.5	249.4 ± 78.6∞
	Hypoxia (45mmHg)	281.8 ± 96.1*	310.2 ± 103.0*	298.1 ± 83.8*	294.7 ± 84.0 *∞
P value		Pre vs Post P=0.05; O_2 P<0.05; Interaction P=0.34		Pre vs Post P<0.05; O_2 P<0.05; Interaction P=0.91	
ICAv ($cm \cdot s^{-1}$)	**Baseline**	33.6 ± 9.7	39.4 ± 13.7	34.7 ± 12.0	37.3 ± 10.7
	Hypoxia (45mmHg)	40.8 ± 13.4	43.8 ± 14.3	43.1 ± 17.7*	45.4 ± 15.2*
P value		Pre vs Post P=0.06; O_2 P=0.05; interaction P=0.26		Pre vs Post P=0.06; O_2 P<0.05; interaction P=0.82	
ICA diameter (mm)	**Baseline**	5.2 ± 0.59	5.2 ± 0.50	5.2 ± 0.44	5.1 ± 0.50
	Hypoxia (45mmHg)	5.4 ± 0.57*	5.4 ± 0.53*	5.4 ± 0.49*	5.3 ± 0.45*
P value		Pre vs Post P=0.75; O_2 P<0.05; Interaction P=0.96		Pre vs Post P=0.17; O_2 P<0.05; Interaction P=0.84	
ICA CVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	**Baseline**	2.4 ± 0.9	2.7 ± 1.0	2.5 ± 1.1	2.6 ± 1.2
	Hypoxia (45mmHg)	2.9 ± 0.9	3.0 ± 0.9	3.2 ± 1.4*	3.2 ± 1.2*
P value		Pre vs Post P=0.33; O_2 P=0.08; Interaction P=0.06		Pre vs Post P=0.62; O_2 P<0.05; Interaction P=0.35	
MCAv ($cm \cdot s^{-1}$)	**Baseline**	60.2 ± 9.7	58.3 ± 9.2	60.9 ± 6.7	57.9 ± 7.1
	Hypoxia (45mmHg)	67.8 ± 12.7*	65.0 ± 14.1*	66.8 ± 10.2*	69.0 ± 9.8*
P value		Pre vs Post P=0.23; O_2 P<0.05; Interaction P=0.25		Pre vs Post P=0.83; O_2 P<0.05; Interaction P<0.05	

For both between-trial placebo or antioxidant. Significant difference with O_2 is indicated by *. Significant difference between pre versus post is indicated by ∞. Note. - $P_{ET}O_2$ = end-tidal partial pressure of oxygen; VE = ventilation; MAP = mean arterial pressure; SpO_2 = peripheral capillary saturation of oxygen; Q_{ICA} = blood flow through the internal carotid artery; ICAv = velocity of blood through the internal carotid artery; ICA CVC = internal carotid artery cutaneous vascular conductance; MCAv = blood velocity through the middle cerebral artery.

Table 3. Hypercapnic and hypoxic reactivity for ICA following placebo or antioxidants. Delta change between pre and post within both placebo and antioxidant is displayed. Data are presented as mean ± SD.

	Placebo			Antioxidant		
	Pre	**Post**	Δ	**Pre**	**Post**	Δ
ICA (mL/100g/min/mmHg)	5.8 ± 2.0	6.8 ± 2.1	-1.0 ± 1.7	5.7 ± 2.0	5.8 ± 1.9	-0.1 ± 2.0
P value	Condition P=0.25; Pre vs Post P=0.34; Interaction P=0.33					
ICA (%ΔCBF/%Δ$P_{ET}CO_2$)	15.1 ± 4.5	16.9 ± 4.6	1.7 ± 4.9	15.1 ± 5.3	15.0 ± 4.3	-0.1 ± 5.0
P value	Condition P=0.40; Pre vs Post P=0.52; Interaction P=0.45					
ICA (%ΔCBF/-%ΔSpO_2)	1.8 ± 0.6	1.6 ± 0.5	-0.3 ± 0.7	1.5 ± 0.7	1.2 ± 0.8	-0.3 ± 0.5
P value	Condition P=0.46; Pre vs Pot P=0.12; Interaction P=0.96					

Note. – ICA = internal carotid artery; CBF = cerebral blood flow; SpO_2 = peripheral capillary saturation of oxygen.

Table 4. Cerebral vascular, hemodynamics, and respiratory variables upon chronic exposure to high altitude following placebo or antioxidants. All data presented in mean ± SD.

		Placebo	Antioxidant	P value
$PetCO_2$ (mmHg)	**Pre**	29.7 ± 3.1	29.2 ± 2.6	Condition P=0.78; Pre-vs Post P=0.58; Interaction P=0.41
	Post	29.2 ± 3.0	29.3 ± 2.3	
MAP (mmHg)	**Pre**	103.5 ± 10.8	103.2 ± 13.1	Condition P=0.29; Pre-vs Post P=0.77; Interaction P=0.29
	Post	102.0 ± 10.3	105.8 ± 10.3	
SpO_2 (% oxyhemoglobin)	**Pre**	84.5 ± 3.1	83.7 ± 1.8	Condition P=0.90; Pre-vs Post P=1.00; Interaction P=0.11
	Post	83.6 ± 3.3	84.6 ± 3.0	
Q_{ICA} ($mL \cdot min^{-1}$)	**Pre**	364.1 ± 64.6	393.3 ± 93.4	Condition P=0.73; Pre-vs Post P=0.86; Interaction P=0.27
	Post	377.4 ± 73.7	361.9 ± 84.7	
ICAv ($cm \cdot s^{-1}$)	**Pre**	39.6 ± 7.1	39.7 ± 7.8	Condition P=0.87; Pre-vs Post P=0.14; Interaction P=0.83
	Post	37.5 ± 8.1	38.0 ± 8.1	
ICA diameter (mm)	**Pre**	4.9 ± 0.6	5.0 ± 0.5	Condition P=0.97; Pre-vs Post P=0.63; Interaction P=0.18
	Post	5.1 ± 0.4	4.9 ± 0.6	
ICA CVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	**Pre**	3.6 ± 0.5	3.8 ± 1.0	Condition P=096; Pre vs post P=0.76; Interaction P=0.08
	Post	3.7 ± 0.9	3.5 ± 0.9	
Q_{VA} ($mL \cdot min^{-1}$)	**Pre**	228.6 ± 199.1	181.0 ± 155.1	Condition P=0.97; Pre-vs Post P=0.56; Interaction P=0.26
	Post	210.7 ± 181.9	253.9 ± 149.0	
VAv ($cm \cdot s^{-1}$)	**Pre**	19.3 ± 4.0	18.1 ± 3.9	Condition P=0.78; Pre-vs Post P=0.42; Interaction P=0.20
	Post	19.1 ± 3.3	20.7 ± 4.9	
VA diameter (mm)	**Pre**	6.2 ± 2.6	6.0 ± 2.5	Condition P=0.96; Pre-vs Post P=0.54; Interaction P=0.11
	Post	6.3 ± 2.5	6.9 ± 2.2	
VA CVC ($mL \cdot min^{-1} \cdot mmHg^{-1}$)	**Pre**	4.0 ± 3.9	3.1 ± 3.0	Condition P=0.82; Pre vs post P=0.78; Interaction P=0.32
	Post	3.6 ± 3.3	4.0 ± 2.4	
gCBF ($mL \cdot min^{-1}$)	**Pre**	895.6 ± 365.7	835.9 ± 332.7	Condition P=0.65; Pre-vs Post P=0.97; Interaction P=0.43
	Post	875.6 ± 379.5	944.1 ± 323.1	

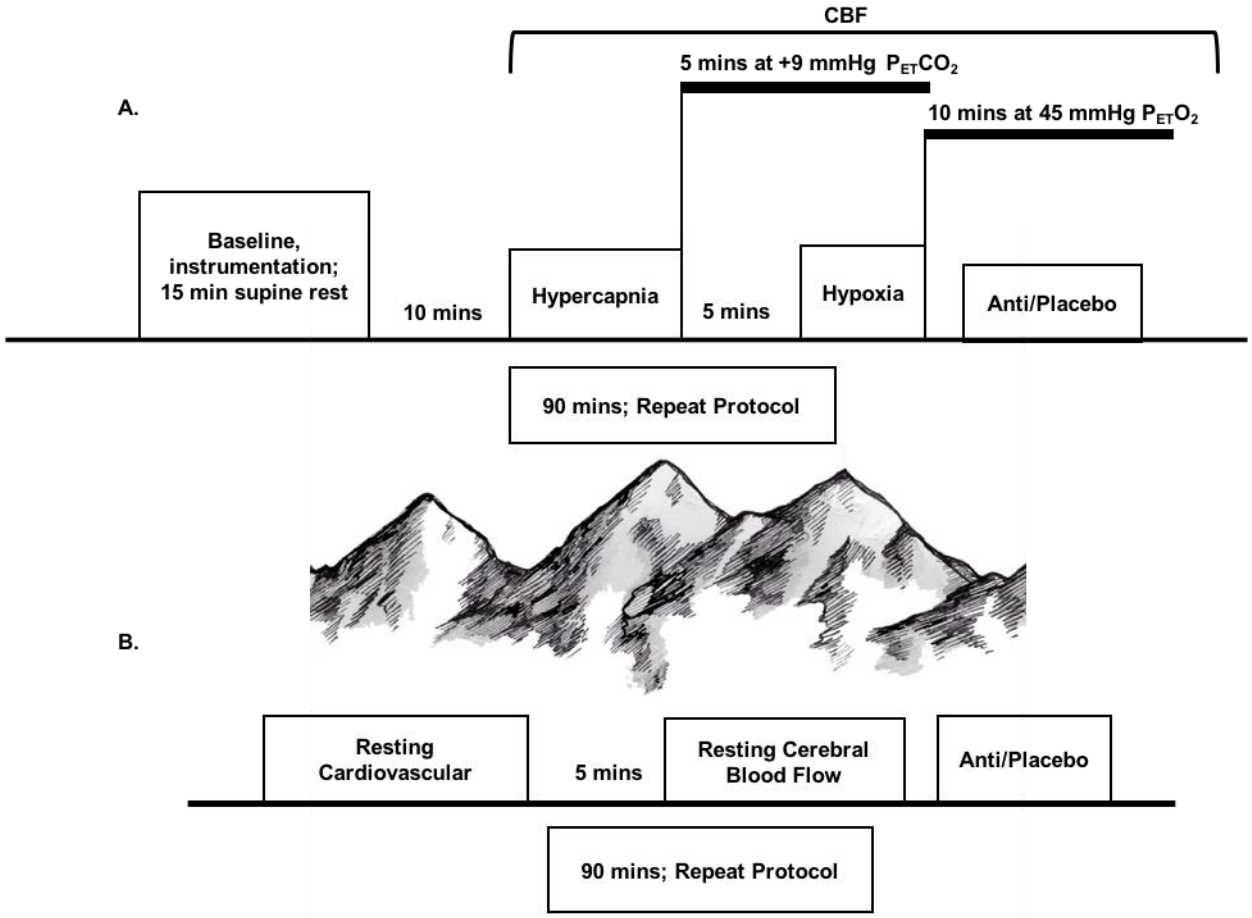
For both between-trial placebo and antioxidant. Note. – $P_{ET}O_2$ = end-tidal partial pressure of oxygen; MAP = mean arterial pressure; SpO_2 = peripheral capillary saturation of oxygen; Q_{ICA} = blood flow through the internal carotid artery; ICA_V = velocity of blood through the internal carotid artery; ICA CVC = internal carotid artery cutaneous vascular conductance; Q_{VA} = blood flow through the vertebral artery; VA_V = blood velocity through vertebral artery; VA CVC = vertebral artery cutaneous vascular conductance; gCBF = global cerebral blood flow.

Figures:

Figure 1. A) Schematic of the experimental protocol for Study 1 representing sea-level (Kelowna, 344 m) measurements & B) Study 2 representing during chronic hypoxia exposure (Evr-K2-CNR pyramid laboratory, 5050 m). Q_{ICA} = blood flow through the internal carotid artery. For hypercapnia end-tidal CO_2 was increased 9 mmHg above baseline resting values. For hypoxia end-tidal O_2 was decreased to 45 mmHg (to simulate arterial PO_2 at precisely 5000m). Resting CV = cardiovascular measurements peripheral capillary saturation of oxygen (SpO_2), end-tidal partial pressure of carbon dioxide ($P_{ET}CO_2$), heart rate (HR), and mean arterial pressure (MAP). Resting CBF = resting cerebrovascular measurements of internal carotid artery (ICA) and vertebral artery (VA).

Figure 2. A) Relative CBF reactivity to hypoxia in the ICA at all experimental trials at sea-level. There was no significant differences in all experimental trials at sea-level (P=0.96). **B) Relative CBF reactivity to hypercapnia in the ICA at all experimental trials at sea-level.** There was no significant differences in al hypercapnic experimental trials at sea-level (P=0.33).

Figure 3. Global cerebral blood flow (gCBF) in all experimental trials after chronic exposure to high-altitude. The combination of both ICA and VA blood flow after chronic exposure to high-altitude. There was no significant differences in all experimental trials at high-altitude (P=0.43).



651
652 **Figure 1.**

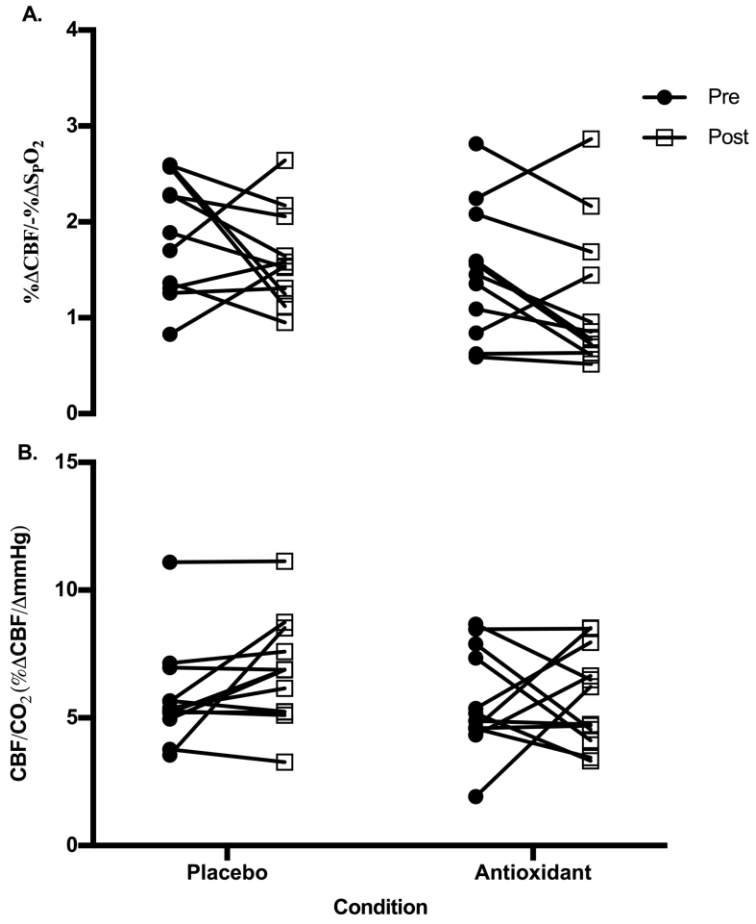

A.
B.
Pre
Post
%ΔCBF/-%ΔSpO2
CBF/CO2 (%ΔCBF/ΔmmHg)
Placebo
Antioxidant
Condition

Figure 2.

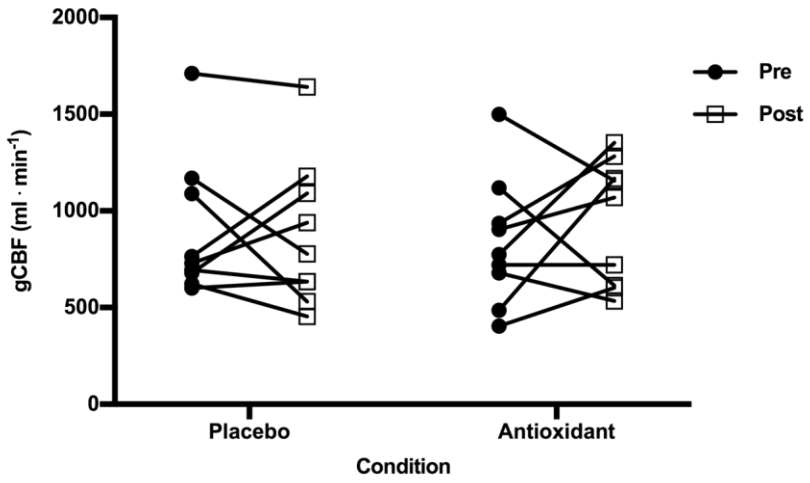

2000
1500
1000
500
0
gCBF (ml · min-1)
Placebo
Antioxidant
Condition
Pre
Post

Figure 3.