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N-Acetyl Cysteine Reduces Hyper-Acute Intermittent Hypoxia Induced Sympathoexcitation in Human Subjects

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Abstract

This investigation tested the hypotheses that: (a) N-Acetyl Cysteine (N-AC) attenuates hyper-acute intermittent hypoxia induced sympathoexcitation, (b) without elevating superoxide measured in peripheral venous blood. Twenty-eight healthy human subjects were recruited to the study. One hour prior to experimentation, each subject randomly ingested either 70 mg·kg⁻¹ of N-Acetyl Cysteine (N-AC, n = 16) or vehicle placebo (n = 12). Three lead electrocardiogram (ECG) and arterial blood pressure (ABP), muscle sympathetic nerve activity (MSNA, n = 17) and whole blood superoxide concentration using electron paramagnetic resonance (EPR, n = 12) spectroscopy were measured. Subjects underwent a 20 minute hyper-acute intermittent hypoxia training (hAIHT) protocol consisting of cyclical end-expiratory apneas with 100% Nitrogen. N-AC decreased MSNA after hAIHT compared to placebo ($P < 0.02$). However, N-AC did not alter superoxide concentrations compared to placebo ($P > 0.05$) in venous blood. Moreover, hAIHT did not increase superoxide concentrations in the peripheral circulation as measured by EPR ($P > 0.05$). Based on these findings, we contend that (a) hAIHT and (b) the actions of N-AC in hAIHT are primarily centrally vs. peripherally mediated, although central measurements of ROS are difficult to obtain in human subjects thus making this assertion difficult to verify. This investigation suggests the possibility of developing a pharmaceutical therapy for inhibiting the sympathoexcitation associated with OSA.

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Competing Interests

No authors declare conflicts of interest.

Author Contributions

PBR, MLS, DWW, WLE and MCZ were involved in experimental design while NPJ, DWW, WLE and GM were involved in data collection. MCZ and JT analyzed and reported EPR data and were blinded to subject IDs, treatment and time point. NPJ analyzed experimental hemodynamic and MSNA data and drafted the manuscript, while all authors reviewed it critically.

Keywords

chemoreflex; sleep apnea; reactive oxygen species; oxidative stress; sympathetic nerve activity; intermittent hypoxia training

Introduction

Obstructive Sleep Apnea (OSA) is associated with a significant increase in cardiovascular morbidity and mortality due to intermittent hypoxia (IH) induced sympathetic activation (Somers *et al.*, 1995; Malpas, 2010). Animal studies have elucidated the causal role of free radicals and reactive oxygen species (ROS) in this phenomenon. The findings of these studies demonstrate that ROS generated at the carotid body, in the nucleus of the solitary tract (NTS) and within the rostral ventral lateral medulla (RVLM) in response to chronic intermittent hypoxia (CIH; i.e. 3–14 days of IH exposure) elicits increases in sympathetic nerve activity (SNA) and hypertension (Peng *et al.*, 2011; Peng *et al.*, 2013; Peng *et al.*, 2014). Further, it has been reported that antioxidant administration substantially reduces the efflux of catecholamines from *ex-vivo* adrenal medullae in rats conditioned to CIH (Kumar *et al.*, 2006). In addition, CIH has been found to reduce the activities of critical antioxidant enzymes in the carotid body, the NTS and RVLM (Peng *et al.*, 2011; Peng *et al.*, 2013; Peng *et al.*, 2014). Human (Foster *et al.*, 2010; Pialoux *et al.*, 2011) and animal (Marcus *et al.*, 2010) studies have demonstrated that oxidative stress is also elevated after acute intermittent hypoxia (AIH; i.e. 4–24 hours of IH exposure), and plays a crucial role in the blood pressure (BP) response to AIH through activation of Angiotensin II Type Ia (AT1a) receptors. It is also known that hyper-acute IH (i.e. <1 hour of IH exposure) alters chemoreflex control of SNA and arterial pressure in human subjects (Morgan *et al.*, 1995; Cutler *et al.*, 2004; Leuenberger *et al.*, 2005); yet, the extent of: (a) the elevation in systemic ROS; and (b) the causal relationship of ROS to elevated SNA is poorly understood in human subjects exposed to hAIH. Animal investigations have reported that 20 minutes to 1 hour of IH produces sensory long-term facilitation (sLTF) of ventilation and enhances neural transmission to central cardiovascular control centers via a superoxide dependent mechanism (Griffioen *et al.*, 2007; MacFarlane & Mitchell, 2008). Furthermore, Yamamoto *et al.* (2015) demonstrated that activating NTS neurons hyper-acutely and intermittently via optogenetic mechanisms produces similar increases in renal SNA compared to hAIH. These findings indicate that hypoxia, chemoreceptor stimulation or peripherally generated oxidative stress is not necessary to produce the sympathetic dysregulation observed with hAIH. Indeed, at least 3 days of IH are required to induce carotid body sensory plasticity and hAIH stimulation is not sufficient to elicit changes in carotid body sensory activity (Prabhakar *et al.*, 2007) These investigations suggest that hyper-acute intermittent hypoxia mediated increases in sympathetic nerve discharge can be reduced with an antioxidant such as N-Acetyl Cysteine (N-AC) (Holdiness, 1991; Hashimoto *et al.*, 2004; Harvey *et al.*, 2008; Jantzie *et al.*, 2010), without altering systemic ROS. The present investigation tested the hypothesis that N-AC, which interacts within the CNS (e.g. NTS and RVLM) tissues, will reduce the sympathoexcitation associated with hyper-acute intermittent hypoxia, while not altering circulating superoxide concentrations measured in the peripheral venous circulation.

Methods

Ethical Approval

The research described herein was approved by the University of North Texas Health Science Center Institutional Review Board (IRB #2011-079). Written informed consent was obtained from all participants in this study in accordance with the Declaration of Helsinki.

Subjects

Twenty-eight human subjects (11 females, 17 males) participated in the protocol. Female subjects were tested during the early follicular phase (days 1–4 post menses) of their menstrual cycle. All subjects completed a medical history questionnaire and physical examination prior to experimentation and were free of cardiovascular, metabolic or respiratory diseases including hypertension. Two subjects were excluded due to mild hypertension and a high pre-test probability of OSA present at the time of experimentation. Both subjects were advised to consult with their primary care physician. Subjects were randomly assigned into placebo (n = 11) or N-Acetyl Cysteine (NAC, n = 15) groups prior to experimentation. Out of these 26 subjects, a reliable MSNA recording was obtained on 17 (N-AC n = 10, Placebo n = 7), and blood samples were successfully obtained on 13 (N-AC n = 6, Placebo n = 7). Hemodynamic data were recorded and analysed for all 26 subjects

Cardiovascular and Hemodynamic Measurements

We measured muscle sympathetic nerve activity (MSNA) with standard microneurographic techniques (n = 17) as described elsewhere (Smith *et al.*, 1996a; Smith *et al.*, 1996b). Briefly, a sterile tungsten microelectrode was inserted into a fascicle of the peroneal nerve near the fibular head. The nerve signals were amplified, filtered (700 to 2,000Hz), rectified and discriminated. The nerve signals were then integrated (time constant = 0.1s) to produce a mean voltage display for quantitative analysis (Jouett *et al.*, 2015; White *et al.*, 2015). Muscle sympathetic neural bursts were readily recognized by their tight temporal relationship to the cardiac cycle, their increasing frequency during Valsalva maneuvers and their failure to respond to arousal stimuli or stroking of the skin (Jouett *et al.*, 2015; White *et al.*, 2015).

A three-lead electrocardiogram (ECG) was obtained (Hewlett-Packard, Inc.) along with beat-to-beat photoplethysmographic measurements of arterial pressure (Finometer, Finapres, The Netherlands). Pulse-oximetry (Nellcor, Inc.) was performed to measure the nadir of O₂ desaturation while ventilation was measured with a Ventilation Measurement Module (VMM, Laguna Hills, CA). All data were digitally recorded in data acquisition software (WinDaq, Dataq, Akron, OH) and stored offline for later analysis.

Measurements of Superoxide in Peripheral Venous Blood

Superoxide concentrations were measured experimentally (n = 13) via electron paramagnetic resonance (EPR) spectroscopy. An intravenous catheter was inserted into an antecubital fossa vein to withdraw 5 mL samples of venous blood. 200 µL of whole blood samples for each time point (pre-ingestion, post-ingestion and hAIHT, see '*Experimental Protocol*' below) were incubated in a buffer solution: 3.5 mM deferoxamine

methanesulfonate salt (DF), 9.08 mM of diethyldithiocarbamic acid sodium (DETC) and Krebs-HEPES buffer (Noxygen Science Transfer & Diagnostics GmbH, Elzach, Germany) containing the superoxide-sensitive methoxycarbonyl-2,2,5,5-tetramethyl-pyrrolidine (CMH) spin probe at 37°C for 15 min (Deo *et al.*, 2012; Vianna *et al.*, 2015). 50 µl samples of whole blood for each time point in duplicate were then loaded into a 1-cc syringe and flash frozen using liquid nitrogen between buffer solutions to form a continuous frozen plug. Samples were then stored at -80°C and shipped to the University of Nebraska Medical Center's EPR Spectroscopy Core for analysis (Deo *et al.*, 2012; Vianna *et al.*, 2015). EPR amplitude was measured using a Bruker e-Scan EPR Spectrometer and was averaged for all duplicate data to generate an individual subject's average for each time point. One subject (placebo) was excluded due to unequal variation among duplicate samples between time points (Levene's test of equal variance $P = 0.004$, final EPR $n=12$).

Experimental Protocol

After instrumentation, baseline MSNA and arterial blood pressure data were obtained for a 5 minute period (pre-ingestion, Figure 1). After the baseline period, a 5 mL blood sample was withdrawn. Subjects then ingested either 70 mg.kg⁻¹ of N-AC (PharmaNAC, BioAdvantex Pharma, Mississauga, Ontario, Canada) dissolved in a fruit-tasting drink as a vehicle or vehicle placebo (same volume of fruit-tasting drink). After a 1 hour ingestion period to reach peak N-AC plasma concentrations (De Caro *et al.*, 1989), another 5 minute baseline period (post-ingestion) commenced where experimental data were recorded and another blood sample draw was performed. Subjects then underwent a 20 minute hAIHT protocol (Figure 1 illustrates 1 minute of the 20 minute protocol). Subjects breathed 2–3 breaths at normal tidal volume of 100% Nitrogen prepared in a Douglas bag, and then initiated a 20-second end-expiratory apnea. Subjects were allowed to recover on room-air for 40 seconds, at which point the N₂ breathing and apnea cycle was repeated and continued for 20 minutes. Desaturations below 90% were targeted for each apnea. The apneic frequency of this protocol approximates severe OSA (AHI ~ 60) for 20 minutes, which is sufficient to elicit significant alterations of sympathetic regulation in human subjects (Cutler *et al.*, 2004). A final 5 mL venous blood sample was obtained immediately after hAIHT to capture the ROS, particularly superoxide, in circulating venous blood post-intermittent hypoxia.

Data Analysis

Data were sampled at 500Hz and recorded directly to a computer with data acquisition software (WINDAQ, Dataq Instruments, Akron, OH). Data were then analyzed using a commercially available biomedical analysis software program (WinCPRS, Absolute Aliens, Turku, Finland). R-waves generated from the ECG were detected and marked at their occurrence in time while diastolic and systolic pressures were marked from the Finometer for arterial blood pressure (Moralez *et al.*, 2012). MSNA bursts were identified by a single investigator and average MSNA burst frequency (bursts/min) and burst incidence (bursts/100 heart beats) were calculated during the entire hAIHT period and the entire baseline periods according to published guidelines (White *et al.*, 2015). Briefly, MSNA bursts were selected by the investigator: if (a) they occurred within 1–1.4 s of an R-wave on ECG; and (b) they exhibited a 3:1 signal-to-noise ratio (White *et al.*, 2015). Hemodynamic and MSNA variables were averaged over the entire 5 minute period to generate averages for the pre-

ingestion and post-ingestion periods. Hemodynamic data during hAIHT were averaged over the last five minutes of the protocol, since it has been identified that hemodynamic variables require at least 15 minutes to increase (Cutler *et al.*, 2004; Leuenberger *et al.*, 2005). In contrast to the increase in MAP the, MSNA increases almost immediately during hAIHT and remains elevated to a similar extent throughout the hAIHT protocol (Cutler *et al.*, 2004). Hence, MSNA was averaged over the entire 20 minute period to generate an hAIHT average for MSNA burst frequency and incidence.

Statistical Analysis

All data were analyzed with commercially available statistical software (SigmaPlot, Systat Software Inc., California, USA). Microneurographic and EPR data were compared for each time point using a three (time point: pre-ingestion, post-ingestion or hAIHT)-by two (treatment: N-AC or placebo) ANOVA, which was followed by Student-Newman-Keuls *post-hoc* tests. Hemodynamic data were first compared pre-ingestion and post-ingestion by Student's t-test, where no statistical differences were observed ($P > 0.05$). Subsequently, a two (time point: post-ingestion or hAIHT)-by two (treatment: N-AC or placebo) ANOVA was performed with subsequent Student-Newman-Keuls *post-hoc* tests. Statistical normality was verified using a Kolmogorov-Smirnov test. Significance was set at $\alpha = 0.05$. Results are reported as means \pm SEM unless otherwise stated.

Results

Subject Demographics and Representative Subject

Subject demographics are provided in Table 1. Placebo control and N-AC subjects did not differ in age, height, weight or body mass index (BMI) (all $P > 0.05$). Furthermore, there were no statistical differences in baseline measures of arterial blood pressure (systolic, diastolic or mean) or HR between placebo and N-AC subjects ($P > 0.05$, Table 1). One minute of experimental data from a representative subject ingesting N-AC for each of the time points are provided in Figure 2. The hAIHT protocol with apnea elicited increases in MSNA as described previously (Smith *et al.*, 1996b) and caused substantial (11%–30%) oxyhemoglobin desaturation.

Hemodynamic Changes During hAIHT and the effect of N-AC on Hemodynamics during hAIHT

Hemodynamic data during the pre-ingestion, post-ingestion and hAIHT periods are provided in Table 2. Hemodynamic variables were not statistically different between pre-ingestion and post-ingestion, hence the arterial blood pressure and heart rate averages were compared from post-ingestion to hAIHT (see 'Data Analysis' and 'Statistical Analysis' sections). Systolic blood pressure (SBP) increased significantly during hAIHT compared to post-ingestion (ANOVA main effect, $P = 0.04$), while mean arterial blood pressure (MAP) trended towards an increase during hAIHT (ANOVA main effect, $P = 0.07$). Diastolic blood pressure (DBP) was unaffected by hAIHT (ANOVA main effect, $P = 0.17$). N-AC significantly reduced SBP and MAP (ANOVA main effect, $P < 0.03$) and subject ingesting N-AC had decreased SBP and MAP during hAIHT compared to placebo control subjects during hAIHT ($P < 0.05$). N-AC did not affect DBP (ANOVA main effect, $P = 0.30$) and no interaction of treatment (e.g.

N-AC vs. vehicle placebo control) and time point were observed for any form of arterial pressure or heart rate (ANOVA interaction effect, $P > 0.05$). Subjects ingesting N-AC increased HR significantly during hAIHT compared to post-ingestion ($P = 0.05$), while subjects ingesting the vehicle placebo did not change their HR significantly ($P > 0.05$).

Alterations in MSNA during hAIHT and the effect of N-AC on MSNA during hAIHT

HAIHT increased MSNA burst frequency and burst incidence significantly (ANOVA main effect, $P < 0.001$), while N-AC reduced the MSNA response to hAIHT (ANOVA main effect, $P < 0.047$). MSNA burst frequency did not display significant interaction between treatment (i.e. N-AC vs. vehicle placebo) and time point (ANOVA interaction effect, $P > 0.05$). Subjects ingesting the vehicle placebo significantly increased MSNA burst frequency with hAIHT ($P < 0.01$). In contrast, subjects ingesting N-AC did not significantly increase MSNA during hAIHT ($P > 0.05$). Furthermore, MSNA (both burst frequency and incidence) was significantly less after hAIHT in subjects who ingested N-AC compared to subjects who ingested placebo ($P < 0.03$, Figure 3).

Changes in Superoxide Concentrations during hAIHT and N-AC effects

Two-way ANOVA demonstrated no main effects of hAIHT or N-AC and no interaction effects on superoxide concentrations in whole blood (all ANOVA main and interactions effects $P > 0.05$, Figure 4). Furthermore, neither N-AC nor placebo subjects nor all subjects combined had significant changes in blood concentrations of superoxide in response to hAIHT (one-sample t-test, all $P > 0.05$), indicating that hAIHT did not significantly elevate superoxide concentrations in the peripheral venous circulation.

Discussion

This study demonstrated that N-AC attenuated the MSNA response to hAIHT but did so without altering peripherally circulating superoxide concentrations as measured by EPR spectroscopy. Furthermore, peripherally circulating superoxide concentrations did not increase after hAIHT. Therefore, we contend that hAIHT and the actions of N-AC in hAIHT are likely centrally versus peripherally mediated, although we were limited in identifying across-the-brain effects of the antioxidant, as discussed below.

Hemodynamic Responses to hAIHT

Our hAIHT protocol elevated arterial blood pressure in a manner consistent with that of Cutler *et al.* (2004) and Leuenberger *et al.* (2005), who utilized a similar hypoxic apnea protocol to the present study. However, arterial blood pressure during hAIHT has been shown to normalize to baseline shortly after the IH exposure while MSNA remains elevated (Cutler *et al.*, 2004). Animal studies utilizing IH exposures indicate that at least 1 day of IH is required to elicit sustained increases in MAP that persist beyond the IH exposure (Marcus *et al.*, 2009; Mifflin *et al.*, 2015), while human studies suggest that as little as 4–6 hours is required (Foster *et al.*, 2010). Hence, we postulate that sustained arterial blood pressure elevations between hyperacute versus longer IH protocols are likely due to slow elevations in circulating neurohormones, such as endothelin and catecholamines (Kanagy *et al.*, 2001). The increases in HR that we observed only with N-AC during IH are probably baroreflex

mediated, as subjects ingesting N-AC had significantly decreased MAP during IH compared to subjects ingesting vehicle placebo (Table 2).

Sympathetic Responses during hAIHT and the Effect of N-AC

Which structures in the cardiovascular neural and humoral arc mediate the hAIHT-dependent increases in MSNA? Although human studies do not permit us to achieve such a distinction, an intact carotid body (Kumar & Prabhakar, 2007; Semenza & Prabhakar, 2015) and neural chemoreflex arc (Peng *et al.*, 2013; Peng *et al.*, 2014) are required to mediate the hypertension observed in CIH conditioned rodents. However, it has been reported that hyper-acute intermittent optogenetic stimulation of NTS neurons increase renal SNA in a fashion similar to hAIHT (Yamamoto *et al.*, 2015), indicating that neither hypoxia, chemoreceptor stimulation nor peripherally generated oxidative stress are required to produce the sympathetic dysregulation observed with hAIHT. Especially considering that at least 3 days of IH exposure are required to alter carotid body responses to hypoxia (Peng *et al.*, 2003; Prabhakar *et al.*, 2007), it is likely that only central (e.g. NTS) components of the chemoreflex mediate sympathetic responses to hAIHT (Mifflin *et al.*, 2015; Prabhakar *et al.*, 2015).

Furthermore, studies in CIH conditioned rodents have identified that both free radicals and ROS generated in the carotid body and the chemoreflex neural arc mediate the carotid body responses to CIH, thereby, activating SNA (Peng *et al.*, 2011; Peng *et al.*, 2013; Prabhakar *et al.*, 2015; Semenza & Prabhakar, 2015). Other animal studies have demonstrated that antioxidants reduce the efflux of catecholamines from the adrenal medulla (Kumar *et al.*, 2006) and reduce the carotid body response to CIH (Peng *et al.*, 2011; Peng *et al.*, 2013; Peng *et al.*, 2014). Furthermore, Pialoux and colleagues (2011) demonstrate that acute intermittent hypoxia (AIH; i.e. 4–24 hours of IH exposure) in human subjects increase markers of oxidative stress, identifying an AT1Ra dependent mechanism, suggesting that shorter exposures of IH can also increase oxidative stress and arterial blood pressure (Foster *et al.*, 2010). However, we have demonstrated that neither hyper-acute exposure to IH nor N-AC altered peripheral superoxide; yet, N-AC significantly lowered the MSNA response to hAIH. We contend that this finding may be explained by N-AC crossing the blood-brain-barrier (BBB) (Holdiness, 1991; Hashimoto *et al.*, 2004; Harvey *et al.*, 2008) and decreasing efferent SNA without altering peripheral redox balance (Figure 4, since hAIHT did not significantly alter peripheral ROS to begin with). Putatively, N-AC could reduce efferent SNA by rescuing nitric oxide (NO), which is rapidly degraded by superoxide and therefore preserves its important sympathoinhibitory function (Paton *et al.*, 2001; Waki *et al.*, 2003; Zimmerman & Davisson, 2004; Zimmerman & Zucker, 2009).

Indeed, our finding that: (a) hAIHT did not increase circulating superoxide concentrations as measured by EPR spectroscopy; and (b) that N-AC had no effect on superoxide concentrations versus placebo in the peripheral circulation suggest that the sympathetic dysregulation and alterations in redox balance during hAIH are primarily centrally-mediated (Griffioen *et al.*, 2007; MacFarlane & Mitchell, 2008). These findings are supported by the following: 1) changes in carotid body activity require at least 3 days of IH exposure (Peng *et al.*, 2003); and 2) stimulation of NTS neurons alone produce similar changes in MSNA and

following hAIH (Yamamoto *et al.*, 2015). However, one of the limitations of the present study is that superoxide is a highly reactive molecule and likely experiences substantial chemical decay during sample collection, hence, we were limited by time in not exploring other measures of oxidative stress suggested by Pialoux *et al.* (2011).

Conclusions

We conclude from these results that N-AC significantly reduces the MSNA response to hAIHT without altering circulating concentrations of free radicals, particularly superoxide. Moreover, peripherally circulating venous superoxide concentrations do not appear to be increased with hAIHT. Since N-AC crosses the BBB these data may be explained by centrally mediated effects of N-AC consistent with previously reported animal studies. Mechanistically, we suggest that N-AC rescues central NO, thereby, decreasing efferent MSNA (Waki *et al.*, 2003) Thus, these findings indicate that centrally active antioxidants, perhaps in combination with angiotensin receptor blockers (ARBs), can provide sympathoinhibitory benefit in clinical conditions of intermittent hypoxia, such as Obstructive Sleep Apnea (OSA) (Somers *et al.*, 1988; Somers *et al.*, 1995)

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New Findings

What is the central question of this study?

This study evaluated the following central question: Does N-Acetyl Cysteine (N-AC), an antioxidant that readily penetrates the blood-brain-barrier, have the capability to reduce the increase in sympathetic nerve activity (SNA) observed during hyper-acute intermittent hypoxia?

What is the main finding and its importance?

We demonstrate that N-AC decreases muscle SNA in response to hyper-acute intermittent hypoxia vs. placebo control. This finding suggests antioxidants such as N-AC have therapeutic potential in Obstructive Sleep Apnea (OSA).

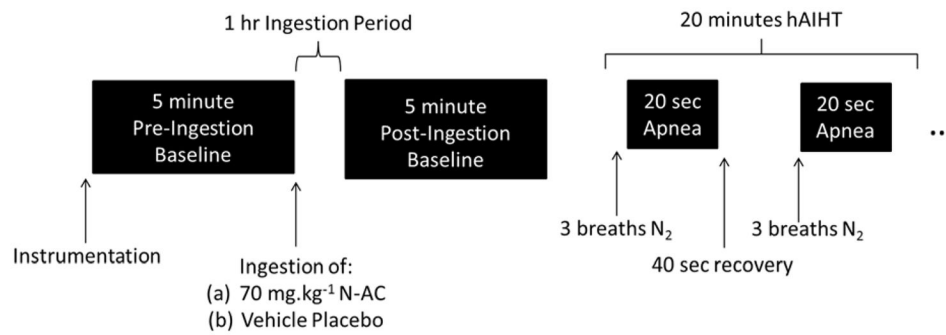


Figure 1.

Representation of the experimental protocol over time. Black boxes represent measurement of hemodynamic, microneurographic and EPR data. Experimental data were averaged over the entire 5 minute period for the pre- and post-ingestion periods. MSNA data were averaged over the entire 20 minute hAIHT period, while hemodynamic data were averaged only during the last 5 minutes of the hAIHT period (see ‘Data Analysis’ for rationale).

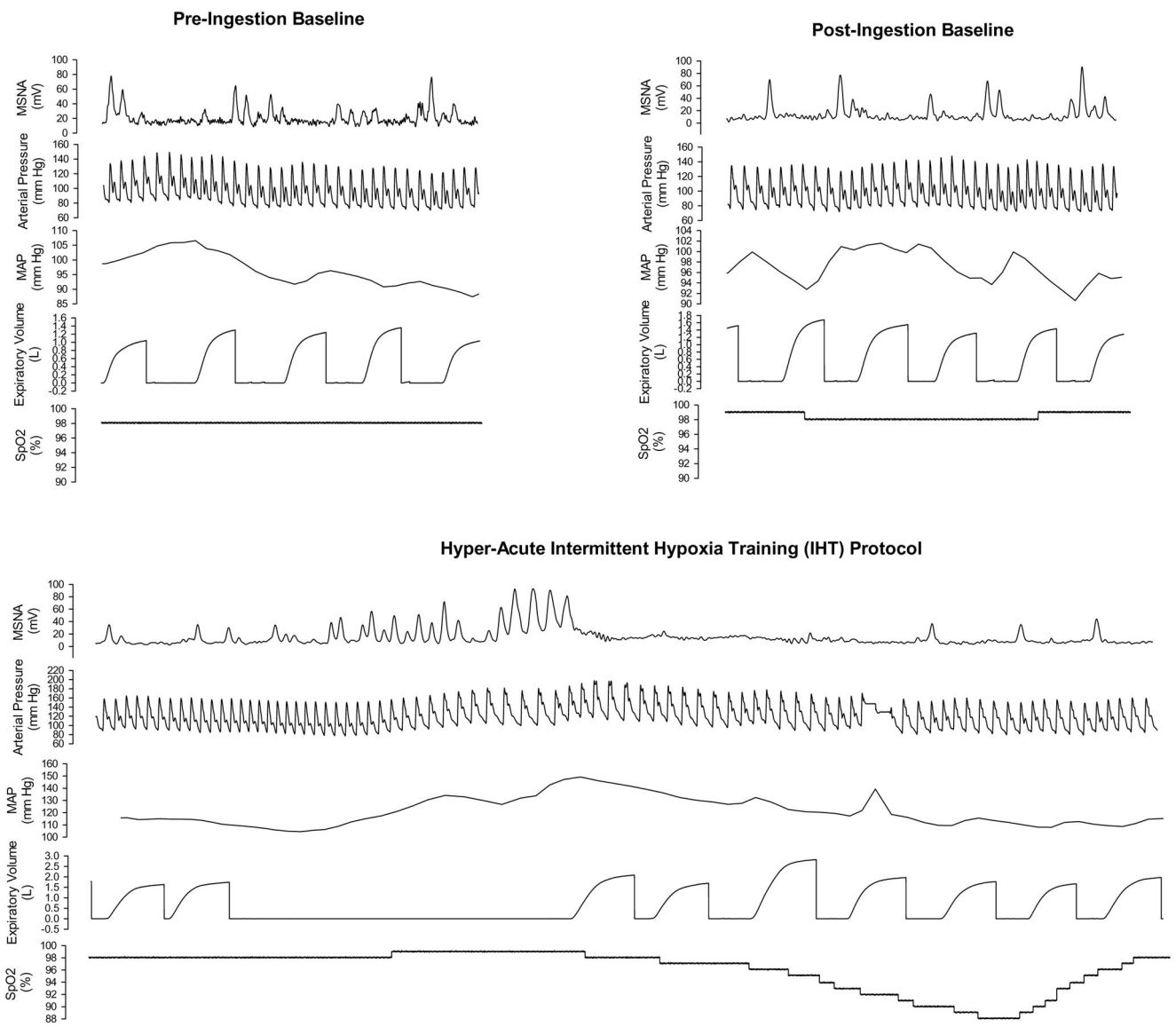


Figure 2. Data from a representative subject ingesting N-AC. Thirty seconds of continuously recorded data from pre-ingestion and post-ingestion baseline periods are provided, while a full cycle (1 minute) of apnea/recovery data are provided in this figure. N-AC did not alter baseline MSNA, however, hAIHT with apnea produced substantial sympathoexcitation. (note: the sudden increase in MAP during the 4th breath after the apnea represents the re-calibration period of the beat-to-beat blood pressure monitor)

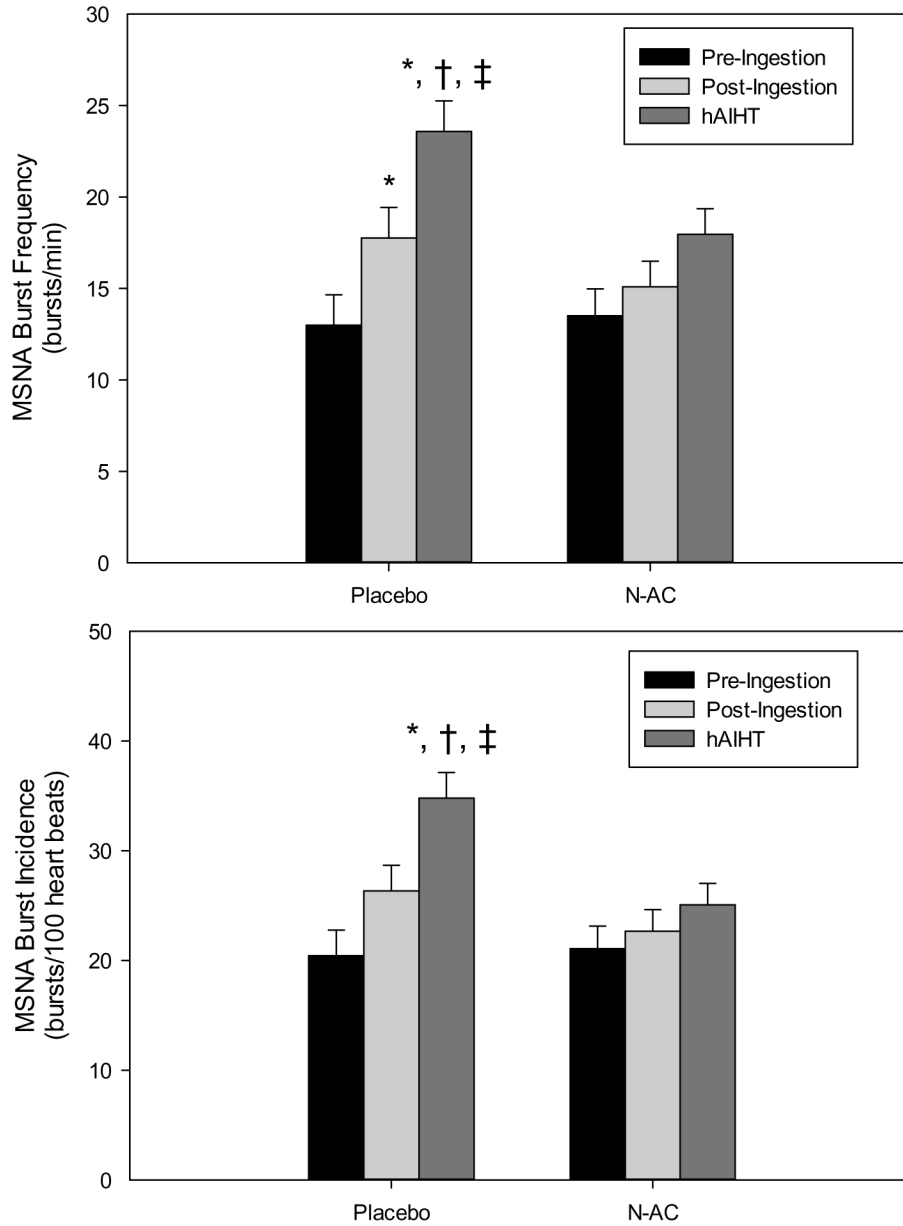


Figure 3. MSNA burst frequency (bursts/minute, top) and incidence (bursts/100 heart beats, bottom) absolute data during the pre-ingestion, post-ingestion and hAIHT time points. *, different from pre-ingestion; †, different from post-ingestion; ‡, different from N-AC corresponding timepoint. N-AC substantially reduced hAIHT sympathoexcitation. MSNA bursts/min: time point, $P < 0.001$; treatment, $P = 0.047$; time point x treatment, $P = 0.15$. MSNA bursts/100 heart beats: time point, $P < 0.001$; treatment, $P = 0.02$; time point x treatment, $P = 0.07$.

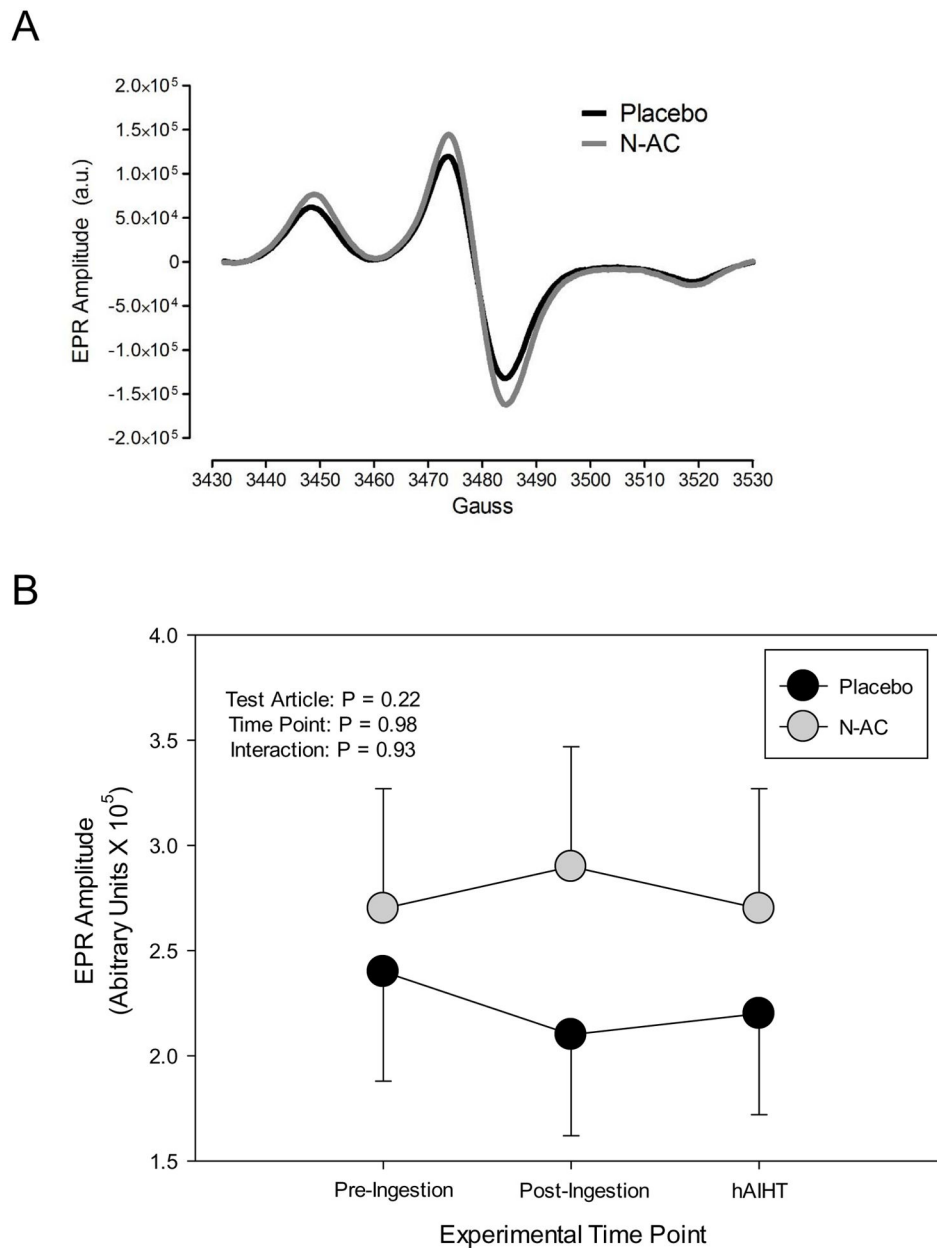


Figure 4. (A) Representative EPR spectra from blood collected from subjects that received either placebo or N-AC. The EPR amplitude (arbitrary units) is directly proportional to the amount of free radicals in the sample. (B) Summary data of EPR amplitude from subjects that received placebo or N-AC at the pre-ingestion, post-ingestion, and hAIHT time-points. ANOVA main effects P values are provided in the top left. Neither hAIHT nor N-AC altered the concentrations of circulating free radicals in peripheral venous blood in the study subjects.

Table 1

Anthropometric and Hemodynamic Variables of Study Sample

Group	Placebo	N-AC	P-Value
<i>Age</i> (years)	32 ± 3	31 ± 3	0.54
<i>Sex</i> (M/F)	6/5	9/6	---
<i>Height</i> (cm)	174.2 ± 3.5	174.5 ± 2.4	0.95
<i>Weight</i> (kg)	83.1 ± 5.9	76.5 ± 3.2	0.30
<i>BMI</i> (kg/m ²)	27.2 ± 1.5	25.1 ± 0.80	0.23
<i>SBP</i> (mm Hg)	129.2 ± 5.2	127.8 ± 3.1	0.81
<i>DBP</i> (mm Hg)	74.4 ± 3.0	72.5 ± 1.6	0.54
<i>MAP</i> (mm Hg)	96.6 ± 3.7	93.2 ± 1.9	0.39
<i>HR</i> (beats per minute)	65.6 ± 2.6	65.3 ± 1.4	0.86

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Table 2

Hemodynamic Data Throughout the Study

Variable	Placebo			N-AC		
	Pre Ingestion	Post Ingestion	HAIHT	Pre Ingestion	Post Ingestion	HAIHT
<i>SBP</i> (mm Hg)	133.9 ± 3.2	132.0 ± 2.9	138.9 ± 2.9	129.3 ± 2.8	127.5 ± 2.1	131.5 ± 2.1 [†]
<i>DBP</i> (mm Hg)	75.5 ± 2.2	73.9 ± 2.2	77.8 ± 2.2	72.5 ± 2.2	73.1 ± 2.1	74.6 ± 1.6
<i>MAP</i> (mm Hg)	99.0 ± 2.8	96.7 ± 2.5	102.3 ± 2.5	93.1 ± 2.4	93.1 ± 1.8	95.8 ± 1.8 [†]
<i>HR</i> (bpm)	64.1 ± 2.6	68.2 ± 3.3	72.3 ± 3.3	65.4 ± 2.3 *	66.0 ± 2.3 *	72.7 ± 2.3

* different from HAIHT, P 0.05

[†] different from placebo condition, P 0.05

Systolic arterial pressure: time point, P=0.04; treatment, P=0.03; time point x treatment, P=0.58

Diastolic arterial pressure: time point, P=0.17; treatment, P=0.30; time point x treatment, P=0.53

Mean arterial pressure: time point, P=0.07; treatment, P=0.03; time point x treatment, P=0.58

Heart rate: time point, P=0.07; treatment, P=0.76; time point x treatment, P=0.65