Effect of Repeated Normobaric Hypoxia Exposures during Sleep on Acute Mountain Sickness, Exercise Performance, and Sleep during Exposure to Terrestrial Altitude

Charles S. Fulco, Stephen R. Muza, Beth Beidleman, Robby Demes, Janet Staab, Juli Jones, Allen Cymerman

Thermal and Mountain Medicine Division

November 2010
U.S. Army Research Institute of Environmental Medicine
Natick, MA 01760-5007

RUNNING HEAD: Normobaric hypoxia pre-treatment and altitude exposure

CORRESPONDING AUTHOR:
Charles S. Fulco, Sc.D.
Thermal and Mountain Medicine Division
USARIEM
Kansas Street
Natick, MA 01760
Charles.fulco@us.army.mil
508 233-4893
508 233-5298 (FAX)
ABSTRACT

There is an expectation that repeated daily exposures to normobaric hypoxia (NH) will induce ventilatory acclimatization (VEacc) and be effective for lessening acute mountain sickness (AMS) and the exercise performance decrement during subsequent hypobaric hypoxia (HH) exposure. However, this notion has not been tested objectively. Healthy, unacclimatized sea level (SL) residents slept for 7.5 hrs each night for 7 consecutive nights in hypoxia rooms under either NH (n=14, 24±5 yr; mean±SD) or “sham” (n=9, 25±6 yr) conditions. The ambient %O₂ for the NH group was progressively reduced by 0.3%O₂ (150 meters equivalent) each night from 16.2%O₂ (2200 m eq) on the 1st night to 14.4%O₂ (3100 m eq) on the 7th night, while that for the ventilatory and exercise-matched sham group remained at 20.9%O₂. Beginning 25 hrs post-sham or NH treatment all ascended and lived for 5 days at HH (4300 m). Partial pressure of end-tidal CO₂ (PetCO₂), oxygen saturation (SaO₂), AMS, and heart rate (HR) were measured repeatedly during daytime rest, sleep or exercise (11.3 km treadmill time trial (TT)). From pre-to post-treatment at SL, resting PetCO₂ decreased (p<0.01) for the NH group (39±3 to 35±3 mmHg) but not for the sham group (39±2 to 38±3 mmHg). Throughout HH, only sleep SaO₂ was higher (80±1% vs 76±1%, P<0.05) and only AMS upon awakening was lower (0.34±0.12 vs 0.83±0.14, P<0.02) in the NH group compared to the sham group; no other between-group rest, sleep, or exercise differences were observed at HH. These results indicate that the VEacc induced by NH sleep was primarily expressed during HH sleep. Under HH conditions, the higher sleep SaO₂ may have contributed to a lessening of AMS upon awakening but had no impact on AMS or exercise performance for the remainder of each day.

KEYWORDS: Ventilatory acclimatization, AMS, physical performance, hypobaric hypoxia, arterial oxygen saturation, SaO₂
Altitude acclimatization results from numerous interrelated physiological adjustments that compensate for hypoxemia, with augmented ventilation being one of the most important and consistently reported (17; 18; 22; 28). Ventilatory acclimatization (VEacc) can be characterized by the progressive decrease in the partial pressure of end-tidal carbon dioxide (PetCO₂) that leads to an increase in arterial oxygen saturation (SaO₂) during the first several days of moderate to high-altitude residence (hypobaric hypoxia [HH; reduced barometric pressure and 20.9% O₂])(7; 28). The enhanced oxygenation is closely linked with reduced acute mountain sickness (AMS) and improved exercise performance during HH residence (1; 11; 12; 14). Some studies show that VEacc can also be induced using 1 to 4 hr HH treatment exposures repeated daily at altitudes ranging from 4300 m to 4500 m in as little as 7 days and are as beneficial as continuous HH residence for reducing AMS and improving exercise performance (2; 4; 18).

A comparable degree of VEacc can likewise be induced as a result of repeated daily normobaric hypoxia treatment exposures (NH; maintained barometric pressure and <20.9% O₂) using many different combinations of hypoxia duration, severity, and frequency (22). What has not been established, however, is whether NH treatment is any more effective than no treatment for mitigating undesirable outcomes such as AMS or the initial large impairment in exercise performance during subsequent HH residence (22). The only controlled, experimental studies reporting that AMS, exercise performance, and other physiological outcomes were affected favorably relative to no treatment utilized either HH treatment prior to HH residence (2-4; 18) or NH treatment prior to NH residence (17; 22). Until two other studies were published recently (5; 27) no data existed to determine directly whether NH treatment would be more beneficial than no treatment during subsequent HH residence.
In one of these studies, our group (5) showed that after inducing \( V_{E\text{acc}} \) using 21 hrs of NH treatment (\( P_O_2 = 90 \text{ mmHg for 2 hrs } \& 110 \text{ mmHg for 1 hr per day} \) over 7 consecutive days the impairment in time-trial exercise performance assessed within a few hrs after rapid ascent to HH (446 mmHg) was not attenuated. The other study (27) used 14 to 18 hrs of NH treatment (12 to 16% \( O_2 \) for 70 to 90 min/d, 3 d/wk, 4 wks) along with an overnight stay at 3611 m and reported no differences in arterial blood gases or AMS when compared to no NH treatment during subsequent HH residence at 4559 m. One interpretation suggested for the lack of effectiveness was a loss of \( V_{E\text{acc}} \) prior to HH residence (5) that resulted from being at sea level without NH treatment for much longer than the \( \leq 24 \text{ hrs} \) used during previous successful HH treatment studies (2; 4). However, this interpretation is inconsistent with the results of at least one study (17) that reported that \( V_{E\text{acc}} \) remained evident when assessed under NH ambient conditions one month after the NH treatment ended. An alternative interpretation for the lack of effectiveness could then be that NH treatment does not provide any additional ventilatory, AMS symptom, or exercise performance benefit during subsequent HH residence.

The main purpose of the current study was to assess the effectiveness of NH treatment \textit{per se} by minimizing the time interval between the end of NH treatment and beginning of HH residence. We included in the shortened time interval both airplane travel and an overnight stopover at a moderate altitude of 2100 m to provide a more realistic scenario that would likely be used by individuals for work or recreational activities. The subsequent HH residence was also lengthened to 5 days to determine whether prior NH treatment would alter the rate of acclimatization.

Our approach was to subject individuals to moderate levels of NH during sleep so that the daily “dose” would be as long as practically possible without interfering with day-time activities.
and also not be so severe to disrupt sleep. This approach also minimized the NH stimulus “down
time” between consecutive treatment exposures (22). To that end, treatment involved sleeping
for 7.5 hrs each night for seven consecutive nights in a room under ambient NH conditions that
simulated progressively increasing altitudes ranging from 2200 m to 3100 m. The total NH
treatment duration was therefore 52.5 hrs, which was nearly twice as long as the minimal total
HH treatment duration previously determined to be beneficial during subsequent HH residence
(5), and approximately three times longer than the two recent NH treatment to HH residence
studies described above (5; 27). We hypothesized that VEacc induced by NH treatment would be
evident, AMS susceptibility would be reduced, and time-trial exercise performance would be
improved compared to a no-treatment control group (“sham”) during the first five days of
residence at a terrestrial elevation of 4300 m.

METHODS

Volunteers. Twenty-three unacclimatized sea-level (SL) residents (20 men and 3 women)
volunteered to participate. None was born at altitudes exceeding 2100 m and all had been living
at low altitudes (<1000 m) for at least 3 months prior to the start of the study. All provided
verbal and written consents after being fully informed of the nature of the study and its possible
risks and benefits. The study was approved by the institutional review boards of the US Army
Research Institute of Environmental Medicine (USARIEM) and the Human Research Protection
Office, US Army Medical Research and Materiel Command.

Experimental design overview. Each volunteer participated in three distinct phases at two
different test facilities over a total period of three to four weeks in the following order (FIGURE
1): [1] a baseline SL assessment phase at USARIEM, Natick, MA (2 weeks, 50 m, P\textsubscript{B} \sim 756 \pm 2
mmHg), [2] a 7-night sleep-treatment phase in Natick, MA, and [3] a 5-day HH phase at the
summit of Pikes Peak, Colorado Springs, CO (4300 m, $P_B \approx 459$ mmHg). During testing in all phases, the temperature was maintained at $21 \pm 3^\circ C$.

**FIGURE 1 Here**

After SL baseline testing was completed but before the sleep-treatment phase began, “squads” of 2 to 4 volunteers were randomly assigned into a NH sleep treatment group (n=14) or a “sham” sleep treatment control group (n=9). Assignment of each volunteer into each squad was based on their availability to travel to Colorado Springs on pre-determined dates. All volunteers were blind to their sleep treatment assignment until the end of the study. No differences between-groups existed for age, weight, height, PetCO$_2$ during rest under SL and NH (1 hr exposure to an ambient PO$_2$ of 93 mmHg) conditions, and peak and time-trial exercise performance (TABLE 1).

**TABLE 1 Here**

During the sleep treatment phase, a squad reported each night at 2200 h to a large room containing two identical 2.4 m width X 3.0 m length X 2.3 m height clear-vinyl sided, portable hypoxia rooms (Colorado Altitude Training, Inc, Boulder CO). One room was always maintained at SL conditions (“sham”, $P_B \approx 756$, $O_2 = 20.9\%$) while the ambient $O_2$ concentration of the other room was progressively reduced by $\sim0.30\%$ $O_2$ (or increased by 150 meter equivalents) on consecutive nights from $\sim16.2\%$ $O_2$ (2200 m eq) on the 1$^{st}$ night to $\sim14.4\%$ $O_2$ (3100 m eq) on the 7$^{th}$ night. Carbon dioxide scrubber units maintained a low concentration of CO$_2$ (0.04% to 0.10%) within each room for all nights. The environmental conditions for the hypoxia rooms were stabilized prior to the volunteers reporting each night. All volunteers

---

1Two days after the sleep treatment phase began a volunteer in the sham group broke a toe (unrelated to the study) and could not participate in the remaining exercise tests, but did participate in all other assessments and activities. Only his resting and sleep data were included in the final analyses.
remained in their room until 0530 hr each morning. Thus all volunteers remained in their respective environmental condition for a total of 7.5 hrs each night.

Between the two adjacent hypoxia rooms was a staff member who each night monitored and controlled the ambient conditions of the hypoxia rooms. Because of the way the tubing, wires, vents, fans and sensors were presented and visually oriented, the volunteers were unaware of O₂ level differences within the rooms.

In the morning after the 7th night of sleep, resting measurements were obtained at SL outside of the hypoxia rooms. All data displays were blinded to the volunteers. Then the volunteers were driven to a local airport and flown to Colorado Springs, CO (2100 m, Pₐ ~600 mmHg) to stay overnight in an apartment under staff supervision. The staff in Colorado was blinded to the volunteers sleep treatment until the entire study was completed. From 0600 h to 0700 h the next morning, the volunteers were driven from the apartment to the summit of Pikes Peak for the 5 day HH phase.

Sleep monitoring. Relative activity during sleep for two nights during the SL baseline phase, 7 nights during the treatment phase, and 4 nights during the HH exposure phase was quantitated for each volunteer by a small motion detector worn on one wrist as previously described (16). Briefly, sleep/awake duration and number of awakenings were determined by motion analyses (Motion Logger with action 4 software, v1.13, Ambulatory Monitoring, Inc. Ardeley, NY).

During sleep, volunteers also wore a small pulse oximeter (Nonin, model 3100, Plymouth MN) on the other wrist that had an adhesive finger sensor that recorded SaO₂, HR, and the number of de-saturation events (>6% drop from baseline for ≥8 sec). To avoid possible variability between device sensitivity, volunteers were assigned the same motion detector and oximeter throughout the study.
Ventilatory measures. Resting ventilation was determined at SL, after 1 hr during NH conditions equivalent to 4300 m (PO2=93 mmHg), at SL in the morning after the last night of sleep treatment, and on days 1, 2, 3, and 5 during HH residence. All resting ventilation tests, as well as pulse oximetry, were done with the volunteers awake, fasting, and rested for at least 30 min. During these procedures, the volunteers were in a seated position while connected to a breathing circuit by a rubber mouthpiece and nose clip, and to a finger pulse oximeter unit (Nonin model 8600, Plymouth MA) to record resting SaO2 and HR. All procedures were performed using a breathe-by-breathe gas analyzer and metabolic measurement system (Vmax 229 Sensormedics Inc, Yorba Linda, CA). The mean PetCO2 value obtained over the last 10 to 15 min of each of the resting ventilation test sessions was the primary variable used to assess VEacc.

Acute mountain sickness. AMS assessments were conducted twice during SL baseline (morning and afternoon); each evening at 2200 h just prior to entering the hypoxia rooms and then every morning at 0530 h before leaving the hypoxia room; at 2100 m just prior to ascending Pikes Peak (0530 h); and then while living at Pikes Peak, 4 times each day for the first 4 days (0700 h [i.e., < 1 hr after awakening], 1400 h, 1700 h, and 2000 h) and twice on the 5th day (0700 h and 1400 h). The prevalence and severity of AMS were determined from information gathered using a shortened version of the Environmental Symptoms Questionnaire (ESQ; (6)). The questionnaire was administered using a personal digital assistant (HP model: iPAQ). A weighted average of scores from 11 symptoms (headache, lightheaded, dizzy, etc.) designated “AMS-C” was calculated. AMS-C scores equal to or greater than 0.7 indicated the presence of AMS. For each day at HH, the AMS-C score obtained at 0700 h and the peak AMS-C score obtained after 0700 were used in the analyses. Prevalence was defined as the percentage of individuals in each group who were sick (i.e., AMS-C ≥ 0.7) at 0700 h and after 0700 h.
At the completion of each AMS assessment, SaO₂ was measured for one min by finger pulse oximetry (Dolphin Medical CO., Voyager Pulse Oximeter, Hawthorne, CA). The mean SaO₂ level was therefore matched in real time to each AMS assessment.

**Peak oxygen uptake (VO₂peak).** One VO₂peak test was conducted during the USARIEM baseline phase at SL. An incremental, progressive exercise bout to volitional exhaustion on a motor-driven treadmill (Model: 9.15HR, Smooth Fitness, King of Prussia, PA) was used to assess VO₂peak. Measurements of VO₂ for each of the two min stages were obtained using a metabolic cart (True Max 2400, Parvo Medics, Sandy, UT). Volunteers walked for a total of 10 min (5 stages) starting at 3 METS (4.8 meters/hr and 0% grade) and ending at 8 METS (6.4 meters/hr and 7% grade). The treadmill speed and grade were then changed so that the volunteers would run at 9.7 meters/hr and 0% (10 METS), respectively. Then every two min stage thereafter, the speed and/or grade were changed such that each successive power output increased by ~1 MET (or 3.5 ml/min/kg). The test continued until VO₂ failed to increase or the volunteer could no longer continue.

**Treadmill Endurance Assessments.** Endurance was determined using a treadmill (Model: 9.15HR, Smooth Fitness, King of Prussia, PA) twice during the USARIEM baseline SL phase and three times during HH residence (days 1, 2, and 5.) The first assessment at SL was used for practice to familiarize the volunteers to the procedures. For all treadmill endurance assessments, there was 5 min of walking at 4.8 meters/hr and 0% grade for warm up followed by 20 min of steady-state exercise at a power output equal to 45 ± 5% of SL VO₂peak. During steady-state exercise, the speed was maintained at 5.6 m/hr and the grade was raised as appropriate (if necessary). For each volunteer, the same speed and grade were used for all steady-state assessments at SL and during HH residence. During the last 5 to 10 min of each 20 min steady-
state exercise session, VO$_2$ was measured using a metabolic cart (True Max 2400, Parvo Medics Salt Lake City, UT). The volunteers were then provided a 5 min break to stretch, use the bathroom, etc.

The volunteers then completed 11.3 kilometers as fast as possible (treadmill time-trial, TT). While the grade remained at 3%, the volunteers could alter the speed to walk or run at any time for any duration during the TT. Volunteers were continuously informed of the distance elapsed but not the time. This type of TT performance test has high test-retest reproducibility and low coefficient of variance, and has been used similarly at altitude (11; 15). Between group changes in TT duration were the primary means to assess whether NH treatment minimized the decrement in exercise performance during HH residence.

Other measures associated with exercise tests. During all exercise tests, heart rate (HR) via HR watch (Polar Electro, Woodbury, NY) was monitored continuously, SaO$_2$ via noninvasive finger pulse oximetry (model 8600, Nonin Medical, Inc, Plymouth, MN) and ratings of perceived exertion (RPE, 6 to 20 Borg scale (8)) were determined either at the end of every work load (during VO$_2$peak) or every five min (during the endurance tests).

Venous blood samples. While seated just prior to exercise at SL and on the mornings (~0800 h to 0900 h) of days 1,2, and 5 during HH residence, 2-ml resting venous blood samples were taken from an arm vein to determine hemoglobin concentration [Hb] and hematocrit (Hct) using an i-STAT portable clinical analyzer (Abbott Point of Care Inc., Princeton, NJ). At SL and on the 2nd (i.e., ~25 hrs after arrival) and 5th mornings in HH, additional 13 ml resting venous blood samples were obtained for the analyses of erythropoietin (Quanatikine IVD ELISA, R&D Systems, Minneapolis, MN), epinephrine and norepinephrine (HPLC, BioRad), and cortisol and aldosterone (EIA, ALPCO Diagnostics, Salem, NH).
Statistical Analyses. Data were analyzed using commercial software (Statistica, ver 7.1 Statsoft, Inc, Tulsa, OK). Two factor (group X time) ANOVAs with repeated measures on one factor (time) were performed on dependent variables (e.g., PetCO₂, SaO₂, etc) that related directly or indirectly to the main hypothesis. In all cases, when significant main effects or interactions were found, Neuman-Kuels post-hoc test was applied. Recent studies of similar experimental procedures using unacclimatized sea-level volunteers were consulted to determine appropriate sample sizes for the major hypothesis related to changes in PetCO₂, AMS symptomatology, and exercise performance (3; 12; 23). It was assumed sham treatment would have no effect on PetCO₂ and that the magnitude of changes for PetCO₂ induced by NH treatment would be similar to that induced by HH treatment of a similar cohort of six volunteers (2). In that study at 4300 m (2; 3), PetCO₂ was reduced by an average of 3.6 ± 2.1 mmHg, AMS was nearly eliminated, and exercise performance was greatly improved by HH treatment. Assuming that NH treatment would similarly reduce PetCO₂ at 4300 m, a minimum of eight volunteers in each group were required to detect a statistically significant between-group difference (alpha <0.05, beta <0.20). Daily differences between groups for AMS prevalence during HH residence were analyzed using Chi-square test for independent groups. Data are presented as mean ± SD. A "P" value of ≤ 0.05 was considered statistically significant for all analyses.

RESULTS

PRIOR TO HYPOBARIC EXPOSURE:

Sleep Monitoring during Treatment. Each night during sleep treatment, SaO₂ was lower (P<0.01) for the NH group than for the sham group with the nightly difference between groups becoming progressively larger from the 1st to the 7th night as the ambient O₂ concentrations for
the NH group progressively decreased. (FIGURE 2). Heart rate did not differ between groups for any night and was maintained at a mean of 64±7 b/min over the 7 nights. For each of the seven nights, both groups experienced identical rates of awakenings (1±1 per night) and similar percentage of being asleep during the time that they were laying down (94±5%), with no change among nights. Also for all nights, the sham group did not experience any desaturation events. For the 1st two nights, the number of desaturation events (< 3 desats / hr) for the NH group did not differ from their SL baseline or from the sham group. However, beginning in the third night (4±4 desats / hr) and continuing through the 7th night (33±33 desats / hr), the number of desaturation events progressively increased for the NH group (P<0.01) and also differed (P<0.01) from the sham group. Lastly, not one person in either group reported AMS on any night during the entire sleep treatment period.

In the morning after the 7th night of sham or NH treatment, each volunteer was asked privately if they thought they slept under SL or NH conditions for the 7 nights. Of the nine who slept under sham conditions, 4 were correct and 5 “had no idea”. Of the 14 who slept under NH conditions, 4 were correct, 3 were wrong, and 7 “had no idea”.

FIGURE 2 Here

Ventilatory Measures Before and Immediately after Sleep Treatment. TABLE 2 shows the results of resting ventilatory assessments for both groups measured during the SL baseline phase, during the acute NH exposure to 4300 m eq. (~1 hr), and in the morning at SL within 2 hrs after awakening from the 7th nightly sleep treatment session. Prior to sleep treatment, there were no differences between groups in any of the measures either at SL or during the <1 hr exposure to NH. Moreover, in general, all values were similarly changed for both groups from SL baseline to acute NH (*P<0.05). At the post-sleep treatment at SL, all values were nearly identical to
their corresponding values measured during SL baseline EXCEPT for a lower value for PetCO2 for the NH group (\(^*P<0.01\)). The PetCO2 value for the NH group also was lower compared to their acute NH PetCO2 value (\(†P<0.05\)) and compared to the sham group during the post-treatment measurement (\(§P<0.01\)). The lower within and between-group PetCO2 value for the NH group indicates that VEacc was successfully induced by the nightly NH treatments.

**TABLE 2 here**

**Travel days.** The volunteers were awakened at \(~0530\) h on the day of travel to Colorado. After the resting ventilatory assessments, volunteers showered, ate breakfast, and were driven to Logan Airport (Boston, MA). Depending on availability, flights departed between 0900 h and 1400 h (median:1200 h) and arrived in Colorado between 1130 h and 1630 h (median:1430 h). The volunteers were then driven to a local apartment (2100 m altitude) where they stayed until \(~0600\) h the next morning when they were driven in \(~1\) hr to the Pikes Peak Laboratory at 4300 m. An interval of \(~25\) hrs occurred between the time the volunteers stepped out the hypoxia rooms in Natick, MA and arrived at Pikes Peak. Within this interval, the volunteers were exposed to \(~21\) hrs of moderate HH conditions (2100 m) that included \(~5\) hrs of air travel and \(~16\) hrs of living in the apartment in Colorado Springs, CO. Just prior to departure from the apartment: resting SaO2 for both groups was similar (\(\sim95\pm3\)%), resting HR for the NH group was lower than that for the sham group (66±10 vs 77 b/min, \(P<0.05\)), and not one volunteer in either group reported AMS at 2100 m.

**EXPOSURE TO HIGH ALTITUDE (HYPOBARIC HYPOXIA):**

**Resting Ventilation.** The between-group difference in resting PetCO2 that existed at SL post-treatment (\(^*P<0.01\)) was no longer detectable during HH residence (**FIGURE 3**). Resting PetCO2 for both groups declined from the 1\(^{st}\) to the 2\(^{nd}\) day (\(~33\) to 31 mmHg, \(P<0.01\)), before
leveling off at ~30 mmHg on days 3 and 5. There also were no differences between groups during HH residence on any day for resting Ve, VO2, VCO2, PetO2, and HR. Arterial oxygen saturation measured concomitantly with resting Ve increased (P<0.01) from acute NH (81±4%) and the 1st two days during HH residence (82±4%) to the 5th day during HH residence (85±5%), but there was no difference between groups on any of the days.

**FIGURE 3 here**

*Daytime Resting SaO2.* The SaO2 data that were independently obtained in conjunction with the ESQ questionnaires were consistent with the SaO2 values collected as part of the resting ventilation assessment. That is, SaO2 increased (P<0.01) for both groups during HH residence from 82±4% on the 1st and 2nd days to 85±5% on the 5th day, with no differences between groups on any of the days during HH residence.

*Sleep Monitoring in HH.* In contrast to a lack of difference in SaO2 between groups while awake during HH residence, the mean sleep SaO2 for the NH group was higher than for the sham group for the entire sojourn (80±4% vs 76±4%, *P<0.05) with nightly between group differences ranging from 2 to 6%. ([FIGURE 4](#)) The NH group also tended to awaken less times than the sham group (12±6 vs 17±7 mean awakenings/night; P=0.06). However, there were no other clear distinctions between groups for all the other variables measured or calculated during sleep (i.e., HR, number of de-saturation events, or awake duration).

From the 1st to the 4th night of sleep during HH residence, for both groups combined, there were declines (P<0.05) in HR (80±10 to 74±7 b/min), number of de-saturation events (333±381 to 201±233 / hr), number of nightly awakenings (17±9 to 11±5), and increases (P<0.05) in sleep SaO2 (76±5 to 81±4%) and %time asleep (76±18 to 84±14%).

**FIGURE 4 here**
Daytime AMS. On the first day, ~80% of the volunteers in each group reported AMS. On the 2nd day, AMS prevalence fell to 29% for the NH group but only to 67% for the sham group (P<0.01). For each of the remaining 3 days, AMS prevalence for both groups became similar. The mean AMS-C severity score was highest for both groups during the 1st day, but then fell rapidly to or below the AMS criteria score of 0.70 for each of the remaining 4 days for both groups (*P<0.01). There were no significant differences between groups for any of the days for AMS-C severity scores during the HH exposure.

AMS Just After Awakening. Figure 5 shows that the prevalence of AMS upon awakening was more than twice as high for the sham group compared to the NH group during the 1st two mornings at HH (P<0.01). For the 3rd and 4th mornings, the prevalence of AMS fell sharply for sham group but remained 8 and 21% higher (P<0.01) compared to the NH group. During HH residence, the mean overall AMS-C severity score upon awakening was higher for the sham group than for the NH group (0.83±0.14 vs 0.34±0.12, *P<0.02). Moreover, only the sham group’s mean AMS-C severity score exceeded the AMS criteria score of 0.70 while under HH conditions (1st two mornings).

FIGURE 5 here

Exercise Assessments: Table 3 shows the responses of Ve, VO2, HR, SaO2, and RPE to the identical, individually determined treadmill speed and grade at SL and during the 1st, 2nd, and 5th days during HH residence. Except for a higher RPE score for the sham group compared to the NH group during HH1, all responses between groups did not differ among the test days. For both groups, from SL to each day during HH, Ve and HR were higher while SaO2 was lower (*P<0.05). For both groups from the 1st to the 5th day during HH, Ve and SaO2 were higher (*P<0.05) while VO2 did not change and did not differ between groups.
Time-trial exercise performance. Time-trial (TT) performance along with HR, SaO₂, and RPE at SL and during HH residence are shown in Table 4. There were no differences between groups for any measure at SL or on any of the three test days during HH except that RPE was higher for the sham group on HH1 (*P<0.05). HR and SaO₂ were reduced and TT performance time increased from SL to each day during HH (*P<0.05). Both groups had a significant improvement in TT performance on HH5 compared to HH1 and HH2 (†P<0.05).

Blood measures. At SL, there were no differences between groups in any of the pre-exercise resting blood values. In addition, on any day during HH exposure, there were no differences between groups for Hb concentration or Hct. However, Hb concentration and Hct were higher on each day during HH compared to SL (*P<0.01).

Erythropoietin (EPO) for both groups increased (*P<0.01) from SL to the 2nd day of HH residence. On the 2nd day, EPO was lower (*P<0.01) for the NH group than for the sham group. Then from the 2nd to the 5th day during HH, EPO declined (bP<0.01) for both groups, and no longer differed from the SL values. However, while under HH conditions, EPO levels remained lower for the NH group than for the sham group (P<0.02).

There were no changes from SL to HH for epinephrine or aldosterone, nor were there any differences between groups on any of the test days. Norepinephrine and cortisol increased (*P<0.01) from SL to the 5th day during HH, but there were no differences between groups.

DISCUSSION

This study tested the hypothesis that ventilatory acclimatization (VEacc) induced by normobaric hypoxia (NH) treatment would be evident under hypobaric hypoxia (HH) conditions
at an altitude of 4300 m and would, in turn, ameliorate AMS symptoms and benefit time-trial (TT) exercise performance. However, there was little indication that VEacc induced by NH sleep treatment was retained while awake during HH residence, and there were no differences relative to the sham group for either AMS (when assessed >1 hr after awakening) or exercise performance outcomes during the five days of residence at 4300 m. In contrast, VEacc was clearly and consistently expressed (via elevated SaO₂) while sleeping in HH and may have contributed to the reduction in AMS and to the attenuated EPO response observed shortly after awakening in HH.

Induction of VEacc has been reported previously during repeated daily exposures to HH or NH treatment (17; 18; 20; 22). Acquisition and retention of VEacc resulting from the repeated HH treatment appears to be an important response associated with reduced AMS symptoms and improved TT performance during subsequent exposure to 4300 m (2-4). In contrast, a significant improvement in SaO₂ induced over a week of 3-hr daily NH treatment exposures was evident only when measured in NH conditions but not when assessed during HH residence at 4300 m, and there was also no improvement in TT performance (5). Reasons for the lack of any retained ventilatory or TT performance benefit during HH residence after NH treatment was considered to be due to a loss of VEacc resulting from the non-treatment time intervals being too long, or to the NH treatment either not inducing sufficient VEacc or simply not being beneficial during subsequent HH residence (5; 27). Based on this information, there was an expectation for the present study that by using a shorter interval of time between the final NH treatment and HH residence as well as by using a NH treatment that was 2.5 to 3.8 times as long as previous NH treatment studies (5; 27) and over two times longer than a highly successful HH treatment (4),
that VEacc would be retained, AMS would be reduced, and TT performance would be improved, during subsequent HH residence.

We were therefore surprised in the present study that after successfully inducing a large VEacc (i.e., -4 mmHg PetCO2) that there were no differences between groups for resting or steady-state exercise ventilatory measures, AMS symptoms (when assessed >1 hr after awakening), or TT performance beginning within a few hours after ascent to 4300 m. The paucity of differences between groups during most of each day while awake as well as the clear difference in SaO2 during sleep at 4300 m were likely not due to controllable, potentially confounding experimental factors for at least a few reasons. First, the similarities between groups during the baseline period in age, weight, height, resting SL and NH PetCO2 values, venous blood values, VO2peak, and TT performance duration prior to sleep treatment minimized the possibility that there would be non-treatment related differences in ventilatory or exercise responses during HH residence. Second, SaO2 was monitored during sham and NH sleep to make certain that the groups were always exposed to and receiving significantly different treatments for each of the seven nights. The sleep oximeters used during the treatments were later used by the same volunteers during HH residence to eliminate possible differences in signal variability between devices. Moreover, SaO2 data were collected independently using different brands of oximeters among the multiple resting and exercise daytime assessments throughout each day to facilitate intrinsic data comparison and result validation. Third, objective measures indicated that significant VEacc was successfully induced by NH treatment and remained after the completion of treatment and on the day of travel. And last, to eliminate possible treatment bias, all volunteers and staff at Pikes Peak were blind to the treatment received by each volunteer and
to the results of all ventilatory, TT performance, and hematological assessments until the entire
study was completed.

Because of these experimental considerations we are confident in stating that VEacc
induced by NH sleep treatment is expressed primarily during sleep but not while awake during
HH residence. Over the four nights at 4300 m, not only were the sleep SaO$_2$ levels significantly
higher for the NH group, but there was also a tendency (P=0.06) for the NH group to awaken less
than the sham group. Moreover, the higher sleep SaO$_2$ observed for the NH group likely
contributed to their lower values for AMS scores ($\leq$ 1 hr after awakening) and the reduced
resting blood EPO levels ($< 3$ hrs after awakening) relative to the sham group during HH
residence. Previous studies reporting a direct relationship between higher SaO$_2$ levels and either
reduced AMS (1; 3) or blood EPO levels (13; 19); or between lower SaO$_2$ levels and increased
AMS (9), are consistent with our interpretation. Whether our findings of apparent sleep response
specificity may be related to possible physiological differences or signaling mechanisms in
response to NH and HH treatments (10; 25) that may be of benefit for the planning of future
acclimatization strategies, cannot be determined from the results of this study.

The daytime resting or exercise absolute values and responses from SL to the initial
assessments during HH residence as well as for the observed rate of acclimatization over the 5
days at 4300 m for PetCO$_2$, SaO$_2$, HR, AMS (other than when just awakened), catecholamines,
and fluid and stress hormones were similar for both groups. All of the values, responses, and
rates of change also were within an expected normal range relative to previous studies that used
similar groups of unacclimatized SL residents who did not undergo any treatment before or while
living under HH conditions (2; 4; 11; 12; 21; 23; 24; 26; 28). Collectively, these results indicate
that there is little justification for using NH treatment prior to HH residence.
Resting PetCO₂ is typically reported to be lower (2; 3; 23; 24) when unacclimatized SL residents are rapidly exposed to HH (e.g., 4300 m, PO₂ ~93 mmHg). In the present study, it was therefore not unexpected that PetCO₂ fell similarly from SL baseline by ~3 mmHg for both groups prior to any experimental treatment in response to the lower ambient PO₂ associated with acute NH conditions (also ~93 mmHg). The ~6 mmHg fall in PetCO₂ for the sham group from SL baseline to the 1st day during HH residence also was anticipated based on previous resting ventilatory data collected on 37 men (24) who were SL residents and likewise rapidly exposed to the identical altitude of 4300 m. We also anticipated that the reduction in PetCO₂ during initial HH exposure would be greater than the reduction observed during acute NH conditions for the same PO₂ of ~93 mmHg based on emerging evidence suggesting ventilatory response differences between NH and HH exposures at the same ambient PO₂ (10).

What was not expected was our finding that the PetCO₂ of the NH group did not remain lower than that of the sham group on any of the five days during HH residence. Previously we had shown that a ~4 mmHg reduction in PetCO₂ (i.e., the same PetCO₂ reduction as observed in the present study) for SL residents undergoing 4 hr daily HH treatments was retained 24 hrs later during subsequent HH residence at 4300 m (446 mmHg; PO₂ = 93 mmHg) (2). Relatedly, in another study (24), PetCO₂ also was ~4 mmHg lower for moderate altitude residents (living at 1600 m) than for SL residents assessed at their respective baseline elevations. When the SL and moderate altitude residents were later assessed while living at 4300 m, PetCO₂ remained ~4 mmHg lower each day for the first five days for the moderate altitude residents compared to the SL residents. The implication for the present study being that if NH treatment was to be as effective as HH treatment during HH residence, the induced ~4 mmHg lower PetCO₂ of the NH group compared to the sham group should have been similarly retained during HH residence.
Why there was no evidence of initial or retained difference for PetCO$_2$ between the NH and sham treatment groups during HH residence remains to be determined. Collectively, in light of our results, experimental design considerations, and at least one study (17) that reported that VEacc induced by NH treatment remained evident for up to 1 month (but only when assessed under NH conditions), a likely and seemingly unavoidable interpretation for a lack of difference between groups for nearly all measures is that NH sleep treatment -- potent enough to have induced significant VEacc -- simply did not provide any additional ventilatory, AMS symptom, or exercise performance benefit while the volunteers were awake during HH residence. In contrast, the NH group did have a significantly higher mean sleep SaO$_2$ compared to the sham group during HH residence that may have contributed to less awakening during sleep, and significantly attenuated AMS NH symptoms and EPO response soon after awakening. Further studies are needed to determine the mechanisms responsible as to why, during subsequent HH residence, that: 1. NH treatment is not nearly as effective as HH treatment and 2. Physiological responses and outcomes resulting from the NH sleep treatment are specific to sleep.

**Perspectives and Significance**

This study clearly shows that NH treatment relative to sham treatment provides little useful benefit during subsequent HH residence. It should be emphasized that the lack of effectiveness of NH treatment was not likely related to an inadequate stimulus or response since the magnitude of the ventilatory acclimatization induced by NH treatment was comparable to that of previous similar studies using HH treatment. In addition, the time interval between the end of NH and later HH residence in the present study was deliberately comparable to that of previous HH treatment-to-HH residence studies. Yet only HH treatment reduced AMS and
improved exercise performance during HH conditions. Interestingly, NH treatment does provide
significant AMS and exercise benefits when the outcome measures are performed under NH
conditions. The most important conclusion resulting from the sum of this all this information is
that NH and HH treatments clearly cannot be used interchangeably and are not equally effective
as pre-acclimatization strategies to reduce AMS and improve exercise performance during
subsequent HH residence.

ACKNOWLEDGEMENTS

This long and involved study could not have been successful without the talents and
sacrifices of many dedicated individuals. The authors would like to thank Paul B. Rock, DO,
Ph.D. who provided 24/7 on-site medical care and COL Keith Hiatt, MD who served as medical
monitor. The authors would also like to thank the following for their hard work at either the
USARIEM or Pikes Peak Laboratory: SGT Michael Cavallo, SSG Jorge Diaz, Leonard D.
Elliott, SGT Sarah M. Elliott, Vincent A. Forte, Jr, SPC Robert Hollins, SGT Mark A. Kryskow,
Eric R. Lammi, Myra L. Reese, SPC Bodunrin G. Shobayo, Ingrid V. Sils, Guy Tatum, and
Richard Viskochil. But most of all, the authors would like to thank all of the volunteers.

DISCLAIMERS

Approved for public release; distribution is unlimited. The views, opinions and/or
findings contained in this publication are those of the authors and should not be construed as an
official Department of the Army position, policy or decision unless so designated by other
documentation. For the protection of human subjects, the investigators adhered to policies of
applicable Federal Law CFR 46. Human subjects participated in these studies after giving their
free and informed consent. Investigators adhered to AR 70-25 and USAMRMC Regulation 70-
25 on the use of volunteers in research. Any citations of commercial organizations and trade
names in this report do not constitute an official Department of the Army endorsement of approval of the products or services of the organizations.
Figure Legends

FIGURE 1. Experimental Design. Resting measures (i.e., ventilation, arterial oxygen saturation (SaO2), and heart rate (HR), venous blood), and exercise determinations of peak oxygen uptake (VO2peak) and endurance performance (steady-state at 45% of VO2peak and a 7 mile time-trial) were obtained during the ~2 week sea level (SL) baseline phase. Also during the baseline phase, resting measures were obtained on all volunteers during an acute <1 hour exposure to NH (12.2% O2, 4300 m equivalent). After being assigned to either the normobaric hypoxia (NH, solid line) or “sham” control sleep (dotted line) treatment group, the volunteers slept for 7 consecutive nights in one of two adjacent and identical 2.4 m X 3.0 m X 2.3 m rooms while being blind to the treatment received. Before (~2200 h) and after (~0530 h) each night of sleep, AMS was assessed, and SaO2 and HR were obtained. Motion, SaO2 and HR were obtained continuously during sleep. Within 2 hours of awakening after the 7th night, post-treatment resting measures were obtained at SL. All were then flown within several hours later to Colorado Springs, CO (2100 m, PB ~600 mmHg) where they resided until ~0600 h the next morning. At ~0700 h, all arrived by car at the summit of Pikes Peak (4300 m).

FIGURE 2. Oxygen Saturation During Normobaric Hypoxia and Sham Treatment. For the sham group, SaO2 remained at ~96% for the entire seven treatment days. In contrast, SaO2 for the NH group began at ~92% and progressively fell over the seven nights to ~88% (*P<0.01). For each of the seven nights, SaO2 for the NH group was lower (*P<0.01) than for the sham group.

FIGURE 3. Ventilatory Acclimatization After Treatment at Sea Level and Pikes Peak. There were no between group differences in resting PetCO2 for any of the test days on the summit of Pikes Peak even though there was a large difference ~25 hours earlier, just after treatment at sea level (aP<0.01). In the 1st morning while residing under hypobaric hypoxia conditions (HH1) PetCO2 had decreased for both groups to a similar value of ~33 Torr with PetCO2 falling more for the Sham group (*P<0.01) than for the NH group (P=0.08). On HH2, PetCO2 continued to fall similarly from HH1 for both groups (aP<0.01) with PetCO2 for each group being lower than their respective post-treatment values (*P<0.01). PetCO2 did not decline further from HH2 to HH3 or HH5 for either group.

FIGURE 4. Oxygen Saturation During Sleep Under Hypobaric Hypoxia Conditions. During sleep for the entire four nights under hypobaric hypoxia conditions, mean SaO2 was higher for the NH treated group compared to the sham group (80±4% vs 76±4%, *P<0.05). For both groups, SaO2 progressively increased from during the 1st to the 4th night of sleep (76±5 to 81±4%, aP<0.05).

FIGURE 5. Symptoms of AMS Upon Awakening Under Hypobaric Hypoxia Conditions. A much larger proportion of volunteers were sick in sham group than in the NH group just after awakening throughout HH. There was an overall difference (*P<0.02) between groups for both AMS prevalence and severity.


### TABLE 1: Volunteer Characteristics

<table>
<thead>
<tr>
<th>Measure</th>
<th>NH Treatment (n=14)</th>
<th>Sham Treatment (n=9)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>24 ± 5</td>
<td>25 ± 6</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 ± 15</td>
<td>75 ± 16</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173 ± 10</td>
<td>174 ± 9</td>
</tr>
<tr>
<td>Gender (M / W)</td>
<td>12 / 2</td>
<td>8 / 1</td>
</tr>
<tr>
<td>Sea Level PetCO2 (mmHg)</td>
<td>39 ± 3</td>
<td>39 ± 2</td>
</tr>
<tr>
<td>Normobaric Hypoxia PetCO2 (mmHg)</td>
<td>36 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Peak Oxygen Uptake (ml/kg/min)</td>
<td>46 ± 8</td>
<td>48 ± 6</td>
</tr>
<tr>
<td>Sea Level Time-Trial Performance (min)</td>
<td>75 ± 13</td>
<td>73 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SD; PetCO2 = Partial pressure of end-tidal carbon dioxide

### TABLE 2. Resting Ventilatory Measures, Before and After Sleep Treatment.

<table>
<thead>
<tr>
<th></th>
<th>SL Baseline</th>
<th>Acute NH</th>
<th>Post-Treat, SL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>NH</td>
<td>Sham</td>
</tr>
<tr>
<td>Ve</td>
<td>8.9±2</td>
<td>8.5±1</td>
<td>9.8±1</td>
</tr>
<tr>
<td>VO2</td>
<td>311±73</td>
<td>296±35</td>
<td>377±78*</td>
</tr>
<tr>
<td>VCO2</td>
<td>234±59</td>
<td>242±32</td>
<td>251±60</td>
</tr>
<tr>
<td>PetO2</td>
<td>101±5</td>
<td>103±6</td>
<td>49±4*</td>
</tr>
<tr>
<td>PetCO2</td>
<td>39.1±1.9</td>
<td>39.1±3.0</td>
<td>36.1±1.7*</td>
</tr>
<tr>
<td>SaO2</td>
<td>97±1</td>
<td>97±1</td>
<td>81±3*</td>
</tr>
<tr>
<td>HR</td>
<td>62±9</td>
<td>64±9</td>
<td>72±12</td>
</tr>
</tbody>
</table>

Values are means±SD; Acute = <1 hr exposure to 4300 m eq; Post-Treat, SL = < 2hrs after awakening after the 7th night of sleep treatment, measured at SL; Sham = Sham control group; NH = normobaric hypoxia group; Ve = resting ventilation (L/min, BTPS); VO2 = Oxygen uptake (ml/min), VCO2 = Carbon dioxide production (ml/min), PetO2 = Partial pressure of end-tidal oxygen; PetCO2 = Partial pressure of end-tidal carbon dioxide; SaO2 = Arterial oxygen saturation (%) ; HR = Heart rate (beats/min). *P<0.05 from within-group SL baseline; †P<0.05 from acute NH exposure; §P<0.01 from Post-Treat Sham.
### TABLE 3. Responses during steady-state exercise at sea level and hypobaric hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ve(BTPS)</td>
<td>37±9</td>
<td>37±7</td>
<td>45±9*</td>
<td>45±11*</td>
<td>43±9*</td>
<td>48±7*</td>
<td>49±9*+</td>
<td>51±11*+</td>
</tr>
<tr>
<td>VO₂</td>
<td>1582±351</td>
<td>1573±307</td>
<td>1604±321</td>
<td>1595±277</td>
<td>1497±324</td>
<td>1652±378</td>
<td>1566±372</td>
<td>1588±318</td>
</tr>
<tr>
<td>HR</td>
<td>129±18</td>
<td>124±7</td>
<td>140±15*</td>
<td>138±7*</td>
<td>138±15*</td>
<td>138±11*</td>
<td>132±12*</td>
<td>134±15*</td>
</tr>
<tr>
<td>SaO₂</td>
<td>97±1</td>
<td>97±1</td>
<td>74±3*</td>
<td>75±4*</td>
<td>73±6*</td>
<td>75±4*</td>
<td>76±9*+</td>
<td>78±4*+</td>
</tr>
<tr>
<td>RPE</td>
<td>8±1</td>
<td>8±1</td>
<td>11±3 a</td>
<td>9±1</td>
<td>11±3</td>
<td>10±4</td>
<td>11±3</td>
<td>10±4</td>
</tr>
</tbody>
</table>

Values are means ± SD; SL = Sea Level, HH1, HH2, HH5 = Hypobaric Hypoxia, days 1, 2, 5; Sham = Sham control group; NH = normobaric hypoxia group; VO₂ = Oxygen uptake (ml/min), Ve = resting ventilation (L/min, BTPS); SaO₂ = Arterial oxygen saturation (%); HR = Heart Rate (beats/min); RPE = ratings of perceived exertion; *P<0.01 from SL; +P<0.05 from HH1; aP <0.05 from HH1 NH RPE.

### TABLE 4: Responses during the time trial performance assessments at sea level and hypobaric hypoxia

<table>
<thead>
<tr>
<th></th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
<th>Sham</th>
<th>NH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR</td>
<td>172±15</td>
<td>165±11</td>
<td>148±18*</td>
<td>152±15*</td>
<td>153±21*</td>
<td>151±19*</td>
<td>149±24*</td>
<td>153±15*</td>
</tr>
<tr>
<td>SaO₂</td>
<td>96±1</td>
<td>97±1</td>
<td>72±6*</td>
<td>74±4*</td>
<td>72±6*</td>
<td>74±7*</td>
<td>73±6*</td>
<td>77±4*</td>
</tr>
<tr>
<td>RPE</td>
<td>13±2</td>
<td>13±2</td>
<td>15±3 a</td>
<td>14±4</td>
<td>15±3</td>
<td>15±4</td>
<td>15±3</td>
<td>13±4</td>
</tr>
<tr>
<td>Time</td>
<td>75±13</td>
<td>73±8</td>
<td>106±21*</td>
<td>103±19*</td>
<td>103±21*</td>
<td>106±22*</td>
<td>99±18+</td>
<td>95±15+</td>
</tr>
</tbody>
</table>

Values are means ± SD; SL = Sea Level, HH1, HH2, HH5 = Hypobaric Hypoxia, days 1, 2, 5; Sham = Sham control group; NH = normobaric hypoxia group; HR = Heart rate (beats/min); SaO₂ = Arterial oxygen saturation (%); RPE = ratings of perceived exertion; Time = Duration (min) to complete the 11.3 meter time trial. *P<0.01 from SL; +P<0.05 from PP1 and PP2; aP <0.05 from HH1 NH RPE.
Baseline (Natick, MA)

SL resting and exercise measures

Acute (< 1 hr) NH exposure
Resting measures (4300 m equivalent)

Oxygen percentage in ambient air = 20.9%

NH Sleep, 7.5 hrs/night (Natick, MA)

Resting ventilatory and exercise measures (5 days)

Pikes Peak (4300 m)

Drive to summit of Pikes Peak (1 hour)

Colorado Springs, CO (Overnight)

Air Travel to Colorado Springs, CO (6 hours)

16.2 15.9 15.6 15.3 15.0 14.7 14.4

Oxygen percentage in ambient air

SLEEP TREATMENT ASSIGNMENT

"Sham" Control Sleep, 7.5 hrs/night (Natick, MA)

Resting ventilatory measures (< 2 hrs post treatment at SL)

0 1000 2000 3000 4000

Altitude or Altitude Equivalent (meters)
Test Day
Post-Treatment HH1 HH2 HH3 HH5
PetCO₂ (mmHg)
28 30 32 34 36 38 40

NH Group
Sham Group

* *, a

#
Night of Sleep

Aterial Oxygen Saturation (%)

NH Group
Sham Group

Night of Sleep

Aterial Oxygen Saturation (%)