Long-Term Facilitation of Ventilation in Humans with Chronic Spinal Cord Injury

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Abstract

Rationale: Intermittent stimulation of the respiratory system with hypoxia causes persistent increases in respiratory motor output (i.e., long-term facilitation) in animals with spinal cord injury. This paradigm, therefore, has been touted as a potential respiratory rehabilitation strategy.

Objectives: To determine whether acute (daily) exposure to intermittent hypoxia can also evoke long-term facilitation of ventilation after chronic spinal cord injury in humans, and whether repeated daily exposure to intermittent hypoxia enhances the magnitude of this response.

Methods: Eight individuals with incomplete spinal cord injury (>1 yr; cervical [n = 6], thoracic [n = 2]) were exposed to intermittent hypoxia (eight 2-min intervals of 8% oxygen) for 10 days. During all exposures, end-tidal carbon dioxide levels were maintained, on average, 2 mm Hg above resting values. Minute ventilation, tidal volume, and breathing frequency

were measured before (baseline), during, and 30 minutes after intermittent hypoxia. Sham protocols consisted of exposure to room air and were administered to a subset of the participants (n = 4).

Measurements and Main Results: Minute ventilation increased significantly for 30 minutes after acute exposure to intermittent hypoxia ($P < 0.001$), but not after sham exposure. However, the magnitude of ventilatory long-term facilitation was not enhanced over 10 days of intermittent hypoxia exposures.

Conclusions: Ventilatory long-term facilitation can be evoked by brief periods of hypoxia in humans with chronic spinal cord injury. Thus, intermittent hypoxia may represent a strategy for inducing respiratory neuroplasticity after declines in respiratory function that are related to neurological impairment. Clinical trial registered with www.clinicaltrials.gov (NCT01272011).

Keywords: intermittent hypoxia; respiration; plasticity

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At a Glance Commentary

Scientific Knowledge on the

Subject: In animal models of spinal cord injury, brief exposure to intermittent hypoxia can elicit a persistent increase in phrenic motor output and improve ventilation. Whether intermittent hypoxia can induce a similar facilitation of ventilation in humans with spinal cord injury remains unknown.

What This Study Adds to the Field:

This study demonstrates that ventilatory long-term facilitation can be evoked in humans with chronic, incomplete spinal cord injury. Single sessions of intermittent hypoxia induced increases in minute ventilation that lasted at least 30 minutes beyond the hypoxia stimulation. These increases occurred within the first 2 days and persisted throughout the 10 days of exposure to intermittent hypoxia, but ventilatory long-term facilitation was not progressively enhanced over this period. This study provides the first evidence that intermittent hypoxia may induce respiratory neuroplasticity in humans with chronic spinal cord injury.

Exposure to intermittent hypoxia (IH) can elicit robust increases in respiratory motor output (1–6) and ventilation (7, 8) that persist beyond stimulus cessation. Evidence suggests this response, termed long-term facilitation (LTF), results from strengthened synaptic inputs to respiratory motoneurons and/or increased motoneuron excitability (1, 9). Three- to 5-minute intervals of hypoxia are sufficient to produce LTF of phrenic motor output in spinal-injured and intact animals. This increased phrenic output after IH persists for more than 60 minutes (3); is more robust after chronic, versus acute, injury (3); and is influenced by the number of hypoxic intervals (1). A daily IH “training paradigm” (7 d) enhances spontaneous and evoked phrenic nerve activity (2) and improves respiratory motor function after cervical spinal cord injury (SCI) in rats (4, 5). However, repeated exposure to IH (7 d) does not enhance diaphragm LTF in spinal-intact rats (10).

IH also evokes ventilatory LTF in neurologically intact humans, but full manifestation of the response requires a slight elevation in end-tidal carbon dioxide levels and is not elicited with hypercapnia alone (7, 8). In healthy control subjects and individuals with obstructive sleep apnea, ventilatory LTF is manifested by a persistent increase in minute ventilation after brief hypoxic episodes (7, 8). Ventilatory responses to IH in humans appear independent of sex (11, 12), but are enhanced after repeated daily exposure (13).

The functional benefits of IH exposure, importantly, may not be limited to the respiratory system. Brief periods of IH also can improve somatic motor function in rats (4) and humans (14) with incomplete SCI. Thus, there is considerable rationale for continued exploration of IH as a novel rehabilitation tool in the context of spinal injury. It is unknown, however, whether the IH-induced LTF of breathing can be evoked after chronic human SCI. The chronically injured spinal cord is in many ways a “new spinal cord” associated with *de novo* propriospinal pathways and altered regulation of motor and autonomic function (15). In addition, compensatory neuroplasticity in spared respiratory motor circuits may impair the ability to express subsequent neuroplasticity after acute IH (16). On the basis of the previous considerations, and the emergence of IH as a possible rehabilitation tool after SCI (14), our primary objective was to test the hypothesis that LTF could be elicited in humans with incomplete, chronic SCI. We predicted LTF would manifest as sustained increases in minute ventilation after acute exposure to IH. Furthermore, we hypothesized that the response magnitude would be enhanced over 10 daily exposures to IH (i.e., a “training effect”). Preliminary results previously were reported in abstract form (17).

Methods

Protocols were approved by the University of Florida and Veterans Affairs Medical Center (Gainesville, FL); and informed consent was obtained. Eleven individuals with incomplete SCI (>1 yr) and upper motor neuron signs, asymptomatic for heart or lung complications, and without tracheostomy or assistive ventilation were recruited. *Incomplete* was defined as some

sensory and/or motor function below the level of injury. This included individuals with American Spinal Injury Association Impairment Scale A (18) when a zone of partial preservation was present. Medical authorization was obtained and participants were assessed by the study physician and therapist. Of 11 individuals enrolled, 1 was ineligible, 2 withdrew, and 8 completed the study (Table 1).

Sham and IH protocols were delivered during wakefulness via a nonrebreathing system and face mask to supine subjects. Physiological measures included end-tidal partial pressures of carbon dioxide (P_{ETCO_2}) and oxygen (P_{ETO_2}) (models 17515 and 17518, respectively; VacuMed, Ventura, CA), sampled from mask ports, and breath-by-breath changes in minute ventilation and its components, breathing frequency and tidal volume, measured via pneumotachography (model RSS100HR-4813; Hans Rudolph, Kansas City, MO). Heart rate and oxygen saturation were monitored via electrocardiogram (model 17032; VacuMed) and pulse oximetry (Biox 3740; Ohmeda, Boulder, CO), respectively.

Exposure to IH was repeated for 10 days (5 d/wk). Participants refrained from caffeine and nicotine for at least 4 hours before each exposure. To enhance recruitment, study visits were limited because of the logistical issues associated with transporting subjects to and from the laboratory. Therefore, on the basis of the negative sham results from previous studies (7), the size of the sham treatment group was kept to a minimum. One-half of the participants ($n = 4$) were blindly administered one sham session 1 day before and after the 10 days of IH. On each day, participants initially breathed room air for 10 minutes to establish eupneic baseline values (baseline 1 [B_1], eupnea). Thereafter, supplemental carbon dioxide was added to the inspirate and P_{ETCO_2} was elevated (2.1 ± 0.4 mm Hg) above B_1 for 10 minutes (baseline 2 [B_2], elevated carbon dioxide) and sustained at this level throughout the protocol. After B_2 , eight 2-minute episodes of 8% oxygen/balance nitrogen were administered; and P_{ETO_2} was maintained at 50 mm Hg using 100% supplemental oxygen. During sham protocols, room air was inspired in place of 8% oxygen. Each hypoxic episode was terminated abruptly with one breath of 100% oxygen to rapidly normalize P_{ETO_2} , followed by a 2-minute recovery period

Table 1: Demographics

ID	Sex	Age (yr)	Height (cm)	Weight (kg)	Duration Post-SCI (yr)	NLI	AIS	UEMS (MS = 50)	LEMS (MS = 50)	Light Touch (MS = 112)	Pin Prick (MS = 112)
1	F	52	165	47.7	6.3	C4	D	46	41	95	47
2	M	61	180	97.7	6.8	T10	D	50	35	87	86
3	M	32	191	94.5	8.0	C4	C	19	7	76	31
4	M	46	191	115.9	4.6	C4	D	37	40	34	20
5*	M	51	188	113.6	2.8	C6	D	50	47	98	99
6*	F	72	160	52.3	4.7	C4	C	10	33	112	112
7*	F	44	157	60.9	2.1	T4	D	50	44	91	72
8*	F	67	168	81.8	5.6	C3	A	50	20	49	31
Mean ± 95% CI		53.1 ± 10.9	175.0 ± 11.8	83.1 ± 22.4	5.1 ± 1.7			39.0 ± 13.3	33.4 ± 11.3	80.3 ± 22.0	62.3 ± 29.1

Definition of abbreviations: AIS = American Spinal Injury Association Impairment Scale; CI = confidence interval; ID = participant identification; IH = intermittent hypoxia; LEMS = lower extremity motor score; MS = maximal score; NLI = neurological level of impairment; SCI = spinal cord injury; UEMS = upper extremity motor score.

*Individuals who were exposed to sham sessions before and after the 10 days of exposure to IH.

breathing room air. Similarly, this single breath of 100% oxygen was provided after each episode of the sham protocols. After the eighth hypoxic/sham episode, physiological measures were monitored for a 30-minute end-recovery (ER) period. All participants tolerated treatment sessions well.

The number and duration of the hypoxic episodes (8% oxygen) used in this study, and the rationale for sustaining elevated levels of carbon dioxide above baseline during wakefulness, were based on previous reports from our group (13), inherent constraints within the SCI population (e.g., desaturation levels), and the desire to demonstrate that relatively short exposures to IH may be sufficient to induce respiratory plasticity and associated improvements in respiratory function (13). Pulmonary function was assessed by digital spirometry, pre- and post-treatment. Figure 1 details the experimental design and protocols.

Data Analysis

Data obtained from two initial (i.e., Days 1 and 2) and two final (i.e., Days 9 and 10) days of the IH protocol, as well as two sham sessions, were analyzed. Breathing during B₁ and B₂ was separated into two 5-minute intervals, and averages of the physiological measures for the last 5 minutes of B₁ and B₂ were determined. The 30-minute ER period was divided into six 5-minute intervals, and physiological measures were averaged for each interval. Single average values were determined for the 30-minute ER period because no obvious differences between the 5-minute intervals were observed. Minute ventilation, tidal

volume, and breathing frequency were expressed as absolute values and fractions of B₂. Measured values from IH protocols were normalized to (1) within-session B₂ values, to determine daily, acute effects; and (2) initial B₂ values, to characterize cumulative effects from repeated (chronic) exposure. For sham protocols, values were normalized to within-session B₂ values. Data from the initial and final sham trials were similar, and therefore, these data were combined for the purpose of statistical analysis and graphical presentation.

A small but significant drift in carbon dioxide levels occurred from B₂ to the ER period (Table 2). To ensure this drift did not explain increases in ventilation observed during the ER period, the minute ventilation change in response to the increase in PETCO₂ from B₁ to B₂ was calculated (Figure 2). On the basis of the measured change, data were fit to a linear curve, and increases in ventilation expected to accompany the drift in carbon dioxide from B₂ to the ER period were calculated. Predicted increases were compared with actual measured values.

Figure 1. Schematic diagram of experimental design and protocols. (A) All participants underwent an initial visit to determine eligibility (visit 1) and 10 days of exposure to intermittent hypoxia (IH). Pulmonary function was tested immediately before and after the first and tenth exposure to IH. Two sham sessions used for control comparisons (one before [Pre-IH Sham] and one after [Post-IH Sham] the 10 d of IH) were completed in a subset of the participants to demonstrate that observed outcomes were associated with exposure to IH, and not placebo or chance. (B) IH and sham protocols are outlined (see METHODS for details). AIS = American Spinal Injury Association Impairment Scale; PETCO₂ = end-tidal partial pressure of carbon dioxide; PETO₂ = end-tidal partial pressure of oxygen; PFT = pulmonary function test.

Table 2: Absolute Values for Physiological Measures during Initial and Final Exposure to Intermittent Hypoxia

	Initial	Final
$\dot{V}_E$		
B ₁ : Eupnea, L/min	10.8 ± 2.7	9.0 ± 1.6
B ₂ : Elevated CO ₂ , L/min	11.6 ± 2.4	10.4 ± 2.2
Predicted ER period, L/min	12.0 ± 2.5	11.4 ± 2.9
Actual ER period, L/min	13.9 ± 2.0*	13.3 ± 2.8*
V_T		
B ₁ : Eupnea, ml	1,141 ± 884	1,096 ± 712
B ₂ : Elevated CO ₂ , ml	1,204 ± 827	1,186 ± 829
ER period, ml	1,282 ± 707	1,335 ± 868*
B _f		
B ₁ : Eupnea, breaths/min	12.9 ± 4.0	14.2 ± 3.7
B ₂ : Elevated CO ₂ , breaths/min	12.8 ± 4.1	14.4 ± 3.2
ER period, breaths/min	13.7 ± 4.1 [†]	15.8 ± 3.8 [†]
P _{ET} O ₂		
B ₁ : Eupnea, mm Hg	114.9 ± 8.1	115.4 ± 3.5
B ₂ : Elevated CO ₂ , mm Hg	118.0 ± 6.5	119.3 ± 4.2
ER period, mm Hg	120.9 ± 4.9	122.5 ± 5.0
P _{ET} CO ₂		
B ₁ : Eupnea, mm Hg	37.1 ± 4.1	39.1 ± 2.2
B ₂ : Elevated CO ₂ , mm Hg	38.9 ± 3.6	40.8 ± 2.4
ER period, mm Hg	39.6 ± 3.4 [†]	41.6 ± 2.0 [†]
SaO ₂		
B ₁ : Eupnea, %	96.8 ± 0.8	96.7 ± 0.8
B ₂ : Elevated CO ₂ , %	97.8 ± 0.7	97.4 ± 1.0
ER period, %	98.0 ± 0.6	97.8 ± 1.1

n = 8.

Shown are the absolute values for minute ventilation ($\dot{V}_E$), tidal volume (V_T), breath frequency (B_f), end-tidal partial pressures of oxygen (P_{ET}O₂) and carbon dioxide (P_{ET}CO₂), and oxygen saturation (SaO₂) during eupneic baseline (B₁), baseline with elevated carbon dioxide (B₂), and end-recovery (ER) period for initial days (initial, i.e., Days 1 and 2) and final days (final, i.e., Days 9 and 10) of the intermittent hypoxia (IH) protocol. Values for $\dot{V}_E$ are reported as predicted (calculated, based on responses to increases in carbon dioxide from B₁ to B₂) and actual values during the ER period. Means ± 95% confidence intervals are reported.

*Significantly different from within-session B₂ values, based on nonparametric analysis.

[†]Significantly different from within-session B₂ values, based on parametric analysis.

Three trials were performed to measure FVC and FEV₁, before and immediately after the 10-day IH protocol. Gains within FVC and FEV₁ were defined as increases that exceeded individual variability detected within repeat measurements at single time points.

Statistical Analysis

Parametric (two-way repeated measures analysis of variance) and nonparametric (Friedman repeated measures analysis of variance on ranks) statistics in conjunction with Student-Newman-Keuls post-hoc tests were employed for normal and nonnormal data, respectively, based on the results of the Shapiro-Wilk method (SigmaPlot 12.5; Systat Inc., Chicago, IL). Reported *P* values are from normalized data and are based on parametric tests unless otherwise denoted, using the symbol ‡, to identify results based on nonparametric tests. Heterogeneity also was assessed, and variances were equal in

all cases where normality was passed. Using the appropriate statistical tests, absolute P_{ET}CO₂ and absolute or normalized measures of minute ventilation, tidal volume, and breathing frequency during B₂ and the ER period on the initial and final days of the protocol were compared. Similarly, absolute P_{ET}CO₂ values during B₁ and B₂ on the initial and final days of the protocol were compared. A paired *t* test was used to compare actual values for minute ventilation during the ER period with predicted values. Likewise, paired *t* tests were used to compare the ventilatory responses during IH versus sham conditions in those four individuals who completed sham protocols, and the absolute P_{ET}CO₂ during B₁ and B₂, and B₂ and the ER period, on days of the sham protocol. Data are presented as means with overlays of individual data points; outliers and 95% confidence intervals (CIs) are identified.

P values less than or equal to 0.05 were considered significant.

Results

Demographics

A heterogeneous group was recruited because the primary purpose of this study was to determine whether ventilatory LTF could be manifested post-SCI. All participants had chronic SCIs, classified as incomplete, and breathed independently. Neurological level of impairment ranged from C3 to C6 for six individuals and from T4 to T10 for two individuals. Injury chronicity ranged from 2 to 8 years. Age, duration postinjury, upper extremity and lower extremity motor scores, and light touch and pin prick scores are presented in Table 1.

Acute Responses from Daily Exposure to IH

Figure 3 illustrates the changes in minute ventilation observed during and after exposure to IH in one participant with C4 SCI. Ventilatory LTF is illustrated by the sustained increase in minute ventilation after IH (Figures 3A and 3D). LTF was successfully induced on both the initial (*P* < 0.001) and final (*P* < 0.001) days of the protocol (Figure 4A and Table 2). Similar increases in ventilation were not induced by the sham protocols (Figure 4A and Table 3). Consequently, minute ventilation after exposure to IH significantly increased compared with respective values during the sham protocols (*P* = 0.03; 95% CI for mean difference [−0.417, −0.0383]).

Figure 2. Effect of carbon dioxide on minute ventilation. Responses in ventilation to the increase in carbon dioxide during baseline (from B₁ to B₂) were used to generate a linear equation (with slope representing ventilatory sensitivity) to determine predicted values of minute ventilation during the end-recovery (ER) period. B₁ = eupneic baseline; B₂ = baseline with elevated carbon dioxide.

Figure 3. Ventilatory long-term facilitation was detected post-spinal cord injury (SCI), during initial (i.e., Days 1 and 2) and final (i.e., Days 9 and 10) days of the intermittent hypoxia (IH) protocol. Shown are breath-to-breath measures of minute ventilation and end-tidal partial pressures of oxygen ($P_{ET}O_2$) and carbon dioxide ($P_{ET}CO_2$) (top to bottom) recorded from one participant (participant 6) with SCI before, during, and after (1) exposure to IH on initial (black, A–C) and final (gray, D–F) days of the protocol and (2) exposure to sham conditions (G–I). Measures from normoxic and isocapnic baseline (B_1 , eupnea) are graphed before the step increase in carbon dioxide, initiated during B_2 (baseline with elevated carbon dioxide). Carbon dioxide was elevated and sustained until the end of the protocols. (A and D) Note that minute ventilation during the end-recovery (ER) period was greater than either baseline (B_1 , eupnea and B_2 , elevated carbon dioxide) after exposure to IH. (G) Data from a representative sham session show that this increase in minute ventilation was absent without hypoxic exposure during the sham protocols.

The increase in minute ventilation during LTF was associated with increases in tidal volume ($P = 0.01$) and breathing frequency ($P = 0.01$) during the ER period (Figures 4B and 4C and Table 2). Compared with the sham protocol, IH resulted in a higher tidal volume during the ER period ($P = 0.02$; 95% CI for mean difference $[-0.285, -0.0523]$). After sham treatment, no significant increases in tidal volume or breath frequency between B_2 and the ER period were detected (Figures 4B and 4C and Table 3).

Cumulative Responses from Repeated Exposure to IH

Cumulative effects of IH over the 10-day protocol were determined by normalizing

minute ventilation, tidal volume, and breathing frequency recorded during the ER period on initial and final days with B_2 values recorded on the initial days of IH exposure (Figures 4D–4F). We detected no changes in the response to IH across the 10 days of exposure. Minute ventilation during B_2 on the final days of the protocol was unchanged compared with initial B_2 values (Figure 4D and Table 2). Likewise, repeated exposure to IH did not enhance minute ventilation during the ER period on the final versus initial days of the protocol (Figure 4D and Table 2). Moreover, tidal volume and breathing frequency during B_2 and the ER period were similar on the initial and final days of the protocol (Figures 4E and 4F and

Table 2). Although responses from repeated exposure to IH were not cumulative, daily increases in minute ventilation remained acutely sustainable across the treatment period ($P < 0.001$) (Figure 4D and Table 2).

Potential Influence of End-Tidal Carbon Dioxide Drift on Ventilation

$P_{ET}CO_2$ during the B_2 period was elevated compared with B_1 , as expected ($P < 0.001$ and $P < 0.05^\dagger$ for experimental and sham protocols, respectively). A small but significant increase in $P_{ET}CO_2$ occurred after exposure to IH, and thus ER period values were elevated compared with B_2 (Table 2) ($P = 0.01$). Therefore, minute ventilation during the ER period was compared with values that were adjusted for the small

Figure 4. (A–C) Acute and cumulative responses resulting from daily and repeated exposure to intermittent hypoxia (IH), compared to sham. Minute ventilation (*top*), tidal volume (*middle*), and breath frequency (*bottom*) during the end-recovery (ER) period at initial (i.e., Days 1 and 2, initial ER period) and final (i.e., Days 9 and 10, final ER period) days of the IH protocol were normalized to values from baseline with elevated carbon dioxide (B_2) within each individual session to characterize daily effects of exposure to IH at the beginning and end of treatment. Data from initial and final sham sessions were normalized in the same way, and averaged because no statistically significant differences between the one sham session immediately preceding (pre-IH-sham) and one sham session immediately after (post-IH sham) the 10 days of hypoxic exposure were detected. (D–F) Values from baseline with elevated carbon dioxide and the ER period during the final days of the protocol (final B_2 and final ER period, respectively) also were normalized to elevated carbon dioxide baseline during initial days of the protocol (initial B_2) to describe the cumulative effects of repeated exposure to IH. Individual data points are graphed and outliers are identified with *diagonal lines* through data points. *Open circles* represent individuals who were exposed to both the IH and sham protocols, and *solid circles* represent individuals exposed only to the IH protocol. *Histograms* represent means, and *error bars* represent 95% confidence intervals. In all graphs, 1.0 represents the baseline to which all data were normalized and compared. *Significantly different from within-session B_2 values; †significantly different from values after IH exposure.

increase in PET_{CO_2} (predicted values, calculated as described in METHODS; Figure 2). The actual values of minute ventilation were significantly greater than the predicted values on both the initial and final days of the IH protocol ($P = 0.02$; 95% CI for mean difference [0.452, 3.280] and $P = 0.004$; 95% CI for mean difference [0.859, 3.131], respectively) (Table 2). During sham protocols, PET_{CO_2} during the ER period remained stable, compared with B_2 .

Pulmonary Function

After the 10 days of IH, FVC and FEV_1 increased in four of the eight individuals

with SCI (Table 4, boldface values). This is in contrast to the three individuals who showed no change, and one individual (participant 3) who demonstrated declines. Of the three individuals showing no improvement, two had thoracic injuries.

Discussion

These data provide the first evidence that IH exposure evokes ventilatory LTF in humans with chronic, incomplete SCIs. Sustained increases in minute ventilation, above baseline, were observed during wakefulness throughout the 10 days of

the protocol. The magnitude of LTF was consistent across the 10-day period, but cumulative responses were not detected.

Methodological Considerations

Ventilatory LTF was detected in all eight participants (100%) with SCI. On the basis of previous studies from our laboratory (7), the manifestation of ventilatory LTF is likely the consequence of exposure to IH in conjunction with elevated carbon dioxide levels. It is possible that facilitation was not induced by hypoxia, but rather by increases in carbon dioxide levels (19, 20). However, this seems an unlikely explanation because, similar to other studies (7, 21), increases after exposure to hypoxia were significant, which was not the case during the sham protocols.

Whether hypoxia per se triggered LTF, or the response resulted from a nonspecific increase in ventilation during hypoxic episodes, should be considered. Evidence from animal models supports the view that hypoxia is the trigger for LTF. For example, respiratory motor activity is facilitated after IH (22–24), but comparable exposures to other stimuli that increase breathing (e.g., hypercapnia, asphyxia) do not evoke the response (25, 26). MacFarlane and colleagues have proposed that hypoxia stimulates brainstem raphe neurons, and the resultant release of serotonin onto respiratory motoneurons initiates an intracellular cascade that ultimately results in respiratory LTF (27).

The effect of factors such as age, sex, injury chronicity, and comorbidities on the magnitude of LTF must also be taken into account. Animal models suggest that age and sex influence LTF (28–30). In addition, cumulative effects from IH exposure have been detected in males with obstructive sleep apnea, using twelve 4-minute episodes of hypoxia with PET_{CO_2} sustained approximately 3 mm Hg above baseline values (13). Therefore, the duration and frequency of hypoxic episodes, level of sustained hypercapnia, and/or presence of sleep apnea (8, 12), which is common after SCI, may also affect the response. The heterogeneity and limited size of our sample preclude any conclusions about the influence of these factors on the magnitude of LTF. It remains evident, however, that ventilatory LTF can be induced by acute IH after chronic damage to the human spinal cord.

Table 3: Absolute Values for Physiological Measures during Sham Sessions

	Pre-IH Sham	Post-IH Sham
V_E		
B ₁ : Eupnea, L/min	9.9 ± 3.2	10.2 ± 2.5
B ₂ : Elevated CO ₂ , L/min	11.2 ± 4.2	11.2 ± 3.5
ER period, L/min	12.0 ± 3.9	11.0 ± 4.9
V_T		
B ₁ : Eupnea, ml	753 ± 235	789 ± 387
B ₂ : Elevated CO ₂ , ml	822 ± 186	809 ± 268
ER period, ml	827 ± 121	780 ± 98
B_f		
B ₁ : Eupnea, breaths/min	14.0 ± 6.3	14.3 ± 7.5
B ₂ : Elevated CO ₂ , breaths/min	14.1 ± 5.9	14.8 ± 6.9
ER period, breaths/min	14.7 ± 4.5	14.6 ± 6.8
$P_{ET_{O_2}}$		
B ₁ : Eupnea, mm Hg	115.0 ± 8.8	115.3 ± 4.3
B ₂ : Elevated CO ₂ , mm Hg	118.2 ± 6.0	118.1 ± 8.1
ER period, mm Hg	119.4 ± 8.0	119.1 ± 10.2
$P_{ET_{CO_2}}$		
B ₁ : Eupnea, mm Hg	37.5 ± 6.3	35.5 ± 7.0
B ₂ : Elevated CO ₂ , mm Hg	39.9 ± 6.2	37.4 ± 6.4
ER period, mm Hg	39.6 ± 6.9	38.0 ± 5.2
Sa_{O_2}		
B ₁ : Eupnea, %	95.4 ± 4.7	96.7 ± 2.2
B ₂ : Elevated CO ₂ , %	95.9 ± 4.6	97.1 ± 2.2
ER period, %	95.8 ± 3.7	97.4 ± 2.3

n = 4.

Shown are absolute values for minute ventilation (V_E), tidal volume (V_T), breath frequency (B_f), end-tidal partial pressures of oxygen ($P_{ET_{O_2}}$) and carbon dioxide ($P_{ET_{CO_2}}$), and oxygen saturation (Sa_{O_2}) during eupnea (B₁), baseline with elevated CO₂ (B₂), and end-recovery (ER) period for the one sham session immediately preceding (pre-intermittent hypoxia [IH] sham) and the one sham session immediately after (post-IH sham) the 10 days of hypoxic exposure. Means ± 95% confidence intervals are reported. There were no statistically significant differences from within-session B₂ values.

A final consideration is the clinical applicability of IH as a rehabilitation strategy. Trumbower and colleagues have established that IH can safely and effectively augment somatic motor function in humans with SCI (14). Our study, however, is the first to address the impact of IH on

respiratory function in the clinical population. Regarding respiratory outcomes, a key consideration is the potential need for mild hypercapnia to enable detection of the LTF response (7). In the current data, the rehabilitative impact of IH (i.e., increased minute ventilation)

required elevated carbon dioxide to be manifest (Figure 2). However, the literature indicates that profound respiratory motor plasticity and recovery occur with longer intervals of poikilocapnic IH training in animal models (4, 5). Thus, it remains possible that longer periods of IH exposure could obviate the need for elevated carbon dioxide. Nevertheless, commercial devices to regulate inspired carbon dioxide are readily available. The RespirAct (Thornhill Research, Toronto, ON, Canada) can stabilize $P_{ET_{CO_2}}$, when fluctuations occur due to phase lags between the fraction of inspired oxygen and $P_{ET_{CO_2}}$ or changes in ventilation. Alternatively, a Pulmanex Hi-Ox mask (Cardinal Health, McGaw Park, IL) and rebreathing circuit can be used to prevent hypocapnia during spontaneous breathing.

Ventilatory LTF (Acute, Daily Responses)

To date, ventilatory LTF has been detected in neurologically intact individuals with (8, 12, 13) and without (8, 11, 12) obstructive sleep apnea. In males and females, the magnitudes of acute ventilatory responses after exposure to IH are similar (11, 12). However, arousal state and presence of obstructive sleep apnea influence response magnitude (12). The current study is the first to demonstrate this phenomenon that directly affects the respiratory system, during wakefulness, in individuals with SCI.

Consistent with findings from Syed and colleagues (12), the ventilatory LTF observed during the protocol was accomplished by significant increases in breathing frequency and tidal volume.

Table 4: FVC and FEV₁ before Initial and after Final Days of Exposure to Intermittent Hypoxia

Participant ID	% Predicted FVC		% Predicted FEV ₁	
	Pre-IH	Post-IH	Pre-IH	Post-IH
1	78.6 (77.8–79.3)	81.8 (81.0–82.6)	52.7 (46.9–56.5)	69.1 (64.9–73.5)
2	75.7 (73.3–76.9)	75.1 (73.3–78.7)	71.8 (53.3–83.3)	71.9 (51.7–85.0)
3	52.0 (51.4–52.5)	43.6 (42.7–45.1)	61.7 (60.2–62.5)	43.3 (39.8–47.6)
4	66.0 (62.0–68.5)	70.7 (70.6–70.8)	62.1 (56.2–66.0)	63.1 (59.7–66.4)
5*	93.4 (91.9–94.5)	102.2 (99.3–103.9)	86.5 (84.7–88.1)	92.0 (88.4–94.7)
6*	13.9 (11.8–16.0)	19.2 (17.5–20.8)	15.5 (13.9–17.0)	21.4 (17.6–25.2)
7*	79.5 (77.8–82.4)	82.8 (79.9–84.8)	81.8 (79.6–84.1)	80.8 (75.6–86.4)
8*	49.5 (43.9–56.6)	74.8 (73.6–75.5)	58.8 (45.9–71.6)	72.1 (71.0–72.6)

Definition of abbreviations: ID = identification; IH = intermittent hypoxia.

Values are reported as the percentage of predicted value based on age, height, weight, and smoking history. Means with (ranges) are reported for three trials per time point.

Boldface values indicate an increase in FVC and FEV₁ after the 10 days of IH.

*Individuals who were exposed to sham sessions before and after the 10 days of exposure to IH.

Previous reports in animal models and humans provide conflicting information on the association between breathing frequency, tidal volume, and ventilatory LTF. Some studies suggest LTF is primarily due to increases in breathing frequency (31–34) whereas others indicate increases in tidal volume are predominant (35, 36).

Repeated Daily Exposure to IH

In animal models, the magnitude of a variety of ventilatory responses is enhanced after repeated exposure to IH (23, 24, 37). More recently, Gerst and colleagues (13) reported that repeated exposure enhanced the magnitude of ventilatory LTF in men with obstructive sleep apnea. Overall, repeated exposure to IH did not enhance responses in the current study. These data are similar to data reported by Terada and Mitchell (10), which indicate diaphragm LTF was not enhanced in spinal-intact rats after 7 days of exposure to IH. Two of the eight individuals in this study, however, were previously diagnosed with obstructive sleep apnea. Like the findings of Gerst and colleagues (13), facilitation was enhanced by repeated IH exposure in these individuals, suggesting that the presence of obstructive sleep apnea enhances the cumulative response.

Further scrutiny of the data indicate that sex may be an important consideration because repeated exposure to IH enhanced ventilatory LTF in all four males, whereas LTF was reduced in all four females. However, it is unclear whether sex differences will be confirmed in a larger sample of individuals with SCI, or whether the presence or absence of obstructive sleep apnea (38) affected our results. An increase

like this in men would be in agreement with the study by Gerst and colleagues (13), but to our knowledge there are no published reports directly examining the effect of repeated exposure on ventilatory LTF in women.

Sex-related differences in response to IH exposure are possible because reports suggest sex hormones play a role in determining the magnitude of LTF. In male rats, LTF is absent after gonadectomy but restored after testosterone replacement (39). Furthermore, in females, the phase of the estrous cycle and levels of progesterone and estrogen influence the magnitude of LTF (28) and ventilatory responses such as arterial PCO_2 , minute ventilation, and the hypoxic ventilatory response (40, 41), respectively. After SCI, low testosterone levels in males (42, 43) and menstrual effects in females (44, 45) are common. Therefore, postinjury alterations in hormones may play a role in determining the magnitude of ventilatory LTF.

Physiological Significance

Approximately 65% of SCIs occur between C4 and T6 (46). A majority of these individuals breathe independently and appear asymptomatic but still suffer some degree of respiratory insufficiency (47–49) that worsens with time (50) and age (51). In chronic animal models of cervical SCI, repeated exposure to IH progressively increases phrenic motor output (3) and improves respiratory function (4, 5). Our findings are the first to suggest that IH also improves ventilatory function in humans with chronic SCI. Cervical and thoracic spinal cord injuries are likely to differentially affect inspiratory versus

expiratory motor function, and thus segmental injury level may alter the respiratory-related outcomes after IH exposure. Interestingly, the two individuals with thoracic injuries showed no IH-induced improvement in FVC and $\text{FEV}_{1.}$ IH may, therefore, more substantially help those individuals with cervical injuries, and in turn, with greater deficits in inspiratory function. In the current data, however, no associations were apparent between the magnitude of LTF and injury duration or impairment in FVC or $\text{FEV}_{1.}$

Whether the outcomes observed resulted from (1) a training effect on the respiratory system due to IH or (2) direct or indirect changes in physiological parameters (e.g., neurotransmitter levels) remains speculative. Interestingly, IH paradigms can induce considerable functional gains in somatic motor systems in animal models (4, 5) and humans (14) with SCI. Thus, now that proof-of-principle has been established regarding breathing, an important next step is to investigate IH dosing for humans and determine additional effects of this protocol on other neural systems and behaviors (e.g., locomotion, cognition, sleep). In addition, elucidating relationships between responses to IH exposure and individual characteristics (e.g., injury level and severity, sex, time postinjury) will be critical. ■

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