Respiratory Physiology & Neurobiology 304 (2022) 103922

Contents lists available at ScienceDirect

Respiratory Physiology & Neurobiology

journal homepage: www.elsevier.com/locate/resphysiol

Exploring inspiratory occlusion metrics to assess respiratory drive in patients under acute intermittent hypoxia

Victoria R. Rodrigues [a,b,1], Wendy L. Olsen [b,d,1], Elaheh Sajjadi [c], Barbara K. Smith [c,d,*], Nicholas J. Napoli [a,b,d,*]

[a] University of Florida, Department of Electrical and Computer Engineering, US
[b] University of Florida, Human Informatics and Predictive Performance Optimization (HIPPO) Lab, US
[c] University of Florida, Department of Physical Therapy, US
[d] University of Florida, Breathing Research and Therapeutics Center, Department of Physiological Sciences, US

ARTICLE INFO

Edited by Mathias Dutschmann

Keywords:
Amyotrophic lateral sclerosis
Acute intermittent hypoxia
$P_{0.1}$
Inspiratory pressure
Occlusion pressure

ABSTRACT

Patients living with Amyotrophic Lateral Sclerosis (ALS) experience respiratory weakness and, eventually, failure due to inspiratory motor neuron degeneration. Routine pulmonary function tests (e.g., maximum inspiratory pressure (MIP)) are used to assess disease progression and ventilatory compromise. However, these tests are poor discriminators between respiratory drive and voluntary respiratory function at rest. To better understand ALS disease progression, we can look into compensatory strategies and how patients consciously react to the occlusion and the effort produced to meet the ventilatory challenge of the occlusion. This ventilatory challenge, especially beyond the $P_{0.1}$ (200 ms and 300 ms), provides information regarding the patient's ability to recruit additional respiratory muscles as a compensatory strategy. Utilizing a standard $P_{0.1}$ protocol to assess respiratory drive, we extend the occlusion time analysis to 200 ms and 300 ms (Detected Occlusion Response (DOR)) in order to capture compensatory respiratory mechanics. Furthermore, we followed an Acute Intermittent Hypoxia (AIH) protocol known to increase phrenic nerve discharge to evaluate the compensatory strategies. Inspiratory pressure, the rate of change in pressure, and pressure generation normalized to MIP were measured at 100 ms, 200 ms, and 300 ms after an occlusion. Airway occlusions were performed three times during the experiment (i.e., baseline, 30 and 60 minutes post-AIH). Results indicated that while AIH did not elicit change in the $P_{0.1}$ or MIP, the DOR increased for ALS patients. These results support the expected therapeutic role of AIH and indicate the potential of the DOR as a metric to detect compensatory changes.

1. Introduction

Amyotrophic Lateral Sclerosis (ALS) is a motor neuron disease that causes paralysis and, eventually, death due to respiratory failure (Kiernan et al., 2011; Zinman and Cudkowicz, 2011. While respiratory motor neuron degeneration leads to a gradual loss of inspiratory pressure-generating capacity (Iżecka, 2003; Singh et al., 2011), resting minute ventilation may be preserved in the early stages of ALS through compensation of less-affected motor neuron pools (e.g., phrenic motor pools). Eventually, inspiratory motor neuron death exceeds the capacity for compensation, causing ventilatory failure and requiring the use of external breathing support (Iżecka, 2003; Bourke et al., 2001; Lechtzin et al., 2002).

To gauge the status and progression of ventilatory compromise in ALS patients, clinicians routinely measure maximal volitional respiratory function, aiming to measure the remaining neuromuscular capacity of breathing. However, these routine clinical measurements may not distinguish between respiratory drive and voluntary function at rest. Differentiating between these two features is critical. Volitional function directs the feed-forward input for neuromuscular recruitment of respiratory muscles. In contrast, respiratory drive will direct the feedback from the brain stem and neurosensory inputs, such as chemoreceptors. This respiratory-driven sensory feedback dictates the internal "need" to breathe and can drive Work of Breathing (WoB). Thus, separating these

* Corresponding authors at: University of Florida, Breathing Research and Therapeutics Center, Department of Physiological Sciences, US.
E-mail addresses: victoria.ribeiro@ufl.edu (V.R. Rodrigues), olsenwendy@ufl.edu (W.L. Olsen), elasajjadi1@gmail.com (E. Sajjadi), bksmith@phhp.ufl.edu (B.K. Smith), n.napoli@ufl.edu (N.J. Napoli).
[1] https://hippo.ece.ufl.edu

https://doi.org/10.1016/j.resp.2022.103922
Received 14 March 2022; Received in revised form 19 May 2022; Accepted 25 May 2022
Available online 6 June 2022

two measurements for ALS patients can depict the demand to breathe: signaled by the brain stem versus the physical volitional ability to recruit respiratory muscle to breathe.

Currently, respiratory drive can be inferred by measuring the electrical activity of respiratory motor neurons (mainly the phrenic nerve in quiet breathing) or the diaphragm's electrical activity. However, this is clinically challenging since these electrical measurements are invasive, time-consuming, and require specialized equipment. An alternative way to estimate respiratory drive is by measuring the static pressure generated by the inspiratory muscles against an occluded airway at 100 ms after the onset of inspiration ($P_{0.1}$) Whitelaw et al. (1975). Because the subject is unable to detect the occlusion before 100 ms, the $P_{0.1}$ can be considered an automatic occlusion response (AOR). This makes the $P_{0.1}$ effort-independent, reproducible, and minimizes vagal influences because pressure swings do not lead to corresponding changes in volume Whitelaw and Derenne (1993). Since the $P_{0.1}$ is a measure of respiratory drive, its amplitude is a function of hypercapnia, changes in central drive, neuromuscular transmission, and the underlying pressure-generating capacity of the respiratory muscles Whitelaw and Derenne (1993). This allows for the attenuation of intra-subject variability between measurements and the removal of the volitional component of respiratory muscle recruitment.

Converse to AOR, a detected occlusion response (DOR) occurs when an inspiratory occlusion is sustained beyond 100 ms, enabling the subject to cognitively detect the occlusion. This detection of the occlusion directs the subject to respond to the inability to obtain the necessary airflow. Thus, the DOR generates a more variable pressure and has clear time-dependent changes within the shape of the pressure waveform, based on the subjects' ability to meet the newly recognized demand of flow. These variable pressure changes could reflect different governing by the central pattern generator of breathing, an altered spinal reflex that intensifies part of the wave, the loss of a muscle that contributes to only one part of the respiratory cycle, or deterioration to a class of motor fibers that are mainly used in one part of the inspiration cycle Whitelaw and Derenne (1993). While prior studies of $P_{0.1}$ provide insights into the AOR, careful study of the DOR may provide additional insights into our knowledge of respiratory drive and compensatory activities of the respiratory muscle pump and whether these can be changed by disease or by therapies to preserve breathing function.

Recently, the ALS community has found both high and low $P_{0.1}$ measurements in subjects who had depressed respiratory function Pinto et al. (2021). A portion of ALS patients are able to meet their alveolar ventilation demand through compensatory respiratory muscle recruitment paradigms, thus lowering their respiratory drive. However, when compensatory respiratory muscle recruitment paradigms cannot be deployed, their respiratory drive increases. Thus, these changes in amplitude within the $P_{0.1}$ are indicators of the variability within compensatory respiratory drive. Additionally, animal studies of ALS illustrate substantial capacity for compensatory plasticity of accessory motor neuron pools to preserve breathing Seven et al. (2018). These changes in compensatory plasticity could potentially preserve respiratory drive and breathing. Further, this capacity for compensatory plasticity suggests that the activation of accessory respiratory muscles may contribute to dynamic pressure generation in the DOR. Therefore, an increase in pressure in the DOR, potentially, suggests compensatory plasticity, providing a stronger recruitment of respiratory muscles. This improved compensatory plasticity is highly desired for ALS patients by prolonging their respiratory capacity.

Current clinical management for ALS prioritizes the preservation of independent breathing for as long as possible. In addition to therapies designed to preserve airway clearance and strengthen the respiratory muscles (Lahrmann et al., 2003; Lange et al., 2006; Bourke et al., 2003; Cheah et al., 2009, a recent study evaluated the effect of a single session of acute intermittent hypoxia (AIH) on breathing in ALS Sajjadi et al. (2022). AIH is a well-characterized stimulus of respiratory plasticity. In this non-invasive intervention, individuals breathe brief episodes of mildly reduced inspired oxygen. Carotid body activation by hypoxia excites brain stem nuclei, leading to an increased rate and depth of breathing, as well as raphe nuclei, which triggers episodic serotonin release. AIH-induced serotonin-mediated cellular signaling on phrenic motor neurons leads to strengthened synaptic inputs to motor neurons Seven et al. (2018), enabling a potential increase in pressure generation in breathing. When compared to administration of normoxia, a single session of AIH led to increased tidal volume and minute-ventilation in both ALS and unaffected controls 60 minutes later but had no effect in maximal inspiratory pressure generation Sajjadi et al. (2022). This leads to our fundamental research question regarding an increase in compensatory plasticity through the induction of AIH by evaluating the pressure at different occlusion times (AOR and DOR) within ALS and healthy controls.

The primary objectives of this study were to (1) compare AOR and DOR of patients with ALS to unaffected age and sex-matched controls and (2) identify whether AIH-induced facilitation of minute ventilation would be accompanied by changes in AOR and DOR. To address these objectives, we imposed a brief, intermittent inspiratory occlusion during resting breathing. We then evaluated absolute pressure, the rate of change of pressure (slope), and pressure normalized by MIP of the subject (P/MIP) at three time points: 100 ms, 200 ms, and 300 ms after the occlusion. The occlusion test was completed at baseline and repeated 30 and 60 minutes after a single AIH session.

2. Methods

2.1. Subjects

Eligible participants included adults aged 21–75 years, diagnosed with bulbar or spinal onset ALS, or age and sex-matched unaffected controls. Eligible adults had a baseline Forced Vital Capacity (FVC) $>$ 60 % predicted for age, sex, and height. ALS participants did not require external breathing support while awake and upright, had an ALSFRS-R score $>$ 33, were not pregnant, and were not currently participating in a clinical trial for ALS-modifying drugs. Study procedures were approved by the Institutional Review Board of the University of Florida and conformed to the standards set by the Declaration of Helsinki. Written informed consent was obtained from all participants. This was a sub-analysis of NCT #03645031.

Twenty-three subjects within the broader study cohort consented to participate and completed the entire study: 10 healthy adult subjects and 13 adults with ALS. Data from four healthy adults and four ALS patients were excluded due to excessive noise and motion artifacts that precluded analysis and interpretation. Thus, we utilized pressure and flow data from 15 participants: six from the control group and nine from the ALS group. Demographic information of the participants is presented in Table 1.

2.2. Study protocol

Screening Session Subjects that consented to participate were subjected to a screening session prior to the beginning of the study. Screening included a detailed medical and pharmacological history, FVC testing and a resting 3-lead ECG. Subjects' FVC were tested while seated upright, according to established recommended guidelines Laveneziana et al. (2019).

Resting Breathing and $P_{0.1}$ Resting breathing and inspiratory occlusion pressure responses were measured at baseline, 30 min post-AIH, and 60 min post-AIH. Patients wore a fitted face mask (V2, Hans Rudolph) connected in series to a heated pneumotach (HR 3813) and a two-way non-rebreathing valve (HR 2700). Participants were tested while sitting upright in a recliner. Flow and pressure data were recorded at a sampling frequency of 1000 Hz using PowerLab (16/30, ADInstruments). After acclimating to the respiratory circuit, a minimum of 5 min of resting breathing was acquired. Then pressure responses were

Table 1
Demographics of study participants.

Subject ID	Cohort	Height (m)	Weight (kg)	BMI	Age	Sex	ALS-FRS/48	FVC (% Predicted)	Onset Type	Use of NIV	Time since diagnosis (years)
1	ALS	1.68	66.67	23.72	68	M	39	103 %	Limb	No	4
2	ALS	1.70	55.51	19.17	59	F	42	113 %	Bulbar	CPAP	1
3	ALS	1.81	81.00	24.75	69	M	38	81 %	Limb	No	6
4	ALS	1.78	71.80	22.66	69	M	40	75 %	Limb	No	10
5	Control	1.62	63.90	24.35	60	F	–	132 %	–	No	–
6	ALS	1.85	86.10	25.04	71	M	34	68 %	Limb	CPAP	2
7	ALS	1.69	75.80	26.63	64	F	42	88 %	Limb	No	1
8	ALS	1.71	75.30	25.75	72	M	40	82 %	Limb	No	1
9	Control	1.70	77.10	26.68	72	M	–	90 %	–	No	–
10	Control	1.82	85.70	26.02	71	M	–	101 %	–	No	–
11	Control	1.71	71.10	24.32	61	M	–	86 %	–	No	–
12	Control	1.59	56.20	22.23	66	F	–	105 %	–	No	–
13	Control	1.72	62.80	21.18	75	M	–	102 %	–	No	–
14	ALS	1.78	98.20	31.06	73	M	35	106 %	Limb	No	2
15	ALS	1.83	84.70	25.29	56	M	45	81 %	Bulbar	No	1

measured during brief inspiratory occlusions. Manual occlusions of the inspiratory port were applied during exhalation and released shortly after inspiratory efforts were visualized by the investigator (within 0.5 s). End Tidal CO_2 ($ETCO_2$) was sampled continuously (Gemini Respiratory Gas Analyzer). MIP was recorded at the end of the $P_{0.1}$ trial. A minimum of three MIP trials were performed until three efforts within 10 % variability were achieved.

Acute Intermittent Hypoxia. After baseline tests, subjects received a single poikilocapnic AIH session. Subjects were seated upright in a recliner for the trial. A commercially available hypoxia generator (Everest Summit II Hypoxico) delivered fifteen, 1-minute bouts of 10 % oxygen to participants through a non-rebreathing facemask, targeting a SpO_2 nadir of 80–85 %. Hypoxic episodes alternated with 2-minute bouts of normoxia with the facemask removed and a return of $SpO_2 > 94$ %. Research personnel applied and removed the facemask from subjects. Vital signs were monitored throughout the AIH sessions. Resting breathing data and $P_{0.1}$ was re-recorded 30 and 60 min after the AIH session.

2.3. Determining occlusion onset & pressure features

The signal's mean for the pressure and flow were removed and filtered with a low pass FIR filter at 15 Hz with 200 dB stopband attenuation to remove unwanted high-frequency noise. To find the start of the airway occlusion, we looked at zero-crossing points for flow and related those points to where the pressure was zero. Points that fit this criterion were compared to peaks at the pressure signal. The closest zero-crossing flow point, before a pressure peak, was defined as the onset of an occlusion. These points were then confirmed with visual inspection. Figure 1 shows the onset of an airway occlusion in the pressure signal. In total, there were 386 airway occlusion occurrences in the data set: 169 from the control group and 217 from the ALS group.

After determining the inspiratory airway occlusion onset, we evaluated inspiratory pressure at three time points: 1) 100 ms after the onset of the airway occlusion ($P_{0.1}$); 2) 200 ms after the onset of the airway occlusion ($P_{0.2}$); 3) and 300 ms after the onset of the airway occlusion ($P_{0.3}$). These points were carefully chosen to track the change in pressure before the subject is aware of the occlusion and moments after the subject detects the inspiratory load. All occlusions were verified to ensure a duration of, at least, 400 ms. At each of these three time points, a rate of change of pressure (slope) value was also calculated ($S_{P_{0.1}}$, $S_{P_{0.2}}$ and $S_{P_{0.3}}$), using the least-squares fit method Golub (1965). These measurements are visually represented in Figure 1. As a way to normalize pressure difference amongst the subjects, we utilized the baseline MIP to calculate the ratio of the pressure between a certain time point and baseline MIP, P/MIP, during the occlusion time. This was achieved by dividing the $P_{0.1}$, $P_{0.2}$ and $P_{0.3}$ by the baseline MIP of the subject, creating $P_{0.1}$/MIP, $P_{0.2}$/MIP and $P_{0.3}$/MIP, respectively.

2.4. Statistical analysis

The features derived from the data were analyzed by means of a mixed-effects model with Cohort (control and ALS), AIH Stage (baseline, 30 min post and 60 min post), and Time After Occlusion (100 ms, 200 ms, and 300 ms) as fixed effects and subject ID as a random effect. Parametric bootstrap was used to assess statistical significance Faraway

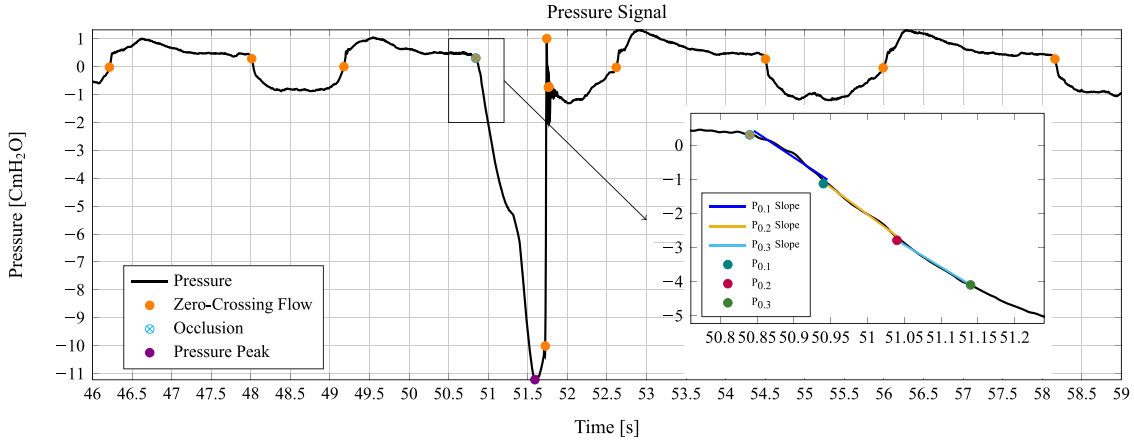

Fig. 1. Representative inspiratory pressure wave during $P_{0.1}$ in an ALS patient. Occlusion points were determined by detecting the zero-crossing points in the flow signal and selecting the zero-crossing point immediately before the negative pressure peak. $P_{0.1}$, P_0. 2 and P_0. 3 were defined as the pressure 100 ms, 200 ms and 300 ms after the occlusion, respectively.

(2016). The criterion of significance was set at $p < 0.05$. To identify any potential drift in $ETCO_2$, values at the first and last minute of each $P_{0.1}$ test were compared using a repeated-measures ANOVA with cell means contrasts. Then, a repeated-measures ANOVA was used to test whether the time post-AIH or subject group affected the $ETCO_2$ recorded during the $P_{0.1}$ trial. Data are reported as mean ± standard deviation. Sperman's correlation between demographic data (Age, Weight, Time after ALS, Onset Type, and Use of NIV) and change in respiratory metrics after AIH ($\Delta P_{0.1}, \Delta P_{0.3}, \Delta S_{P_{0.1}}, \Delta S_{P_{0.3}}, \Delta P_{0.1}$/MIP, $\Delta P_{0.3}$/MIP) was calculated. To further test for correlation between categorical data and change in respiratory metrics, we used the Wilcoxon rank-sum test to test for difference in respiratory metrics between ALS patients using NIV or not and ALS onset type.

3. Results

Tables 1 and 2 display the demographics and standard respiratory measures of the 15 participants, respectively. No significant between-group differences were observed for age ($p = 0.82$), BMI ($p = 0.61$), or weight ($p = 0.23$), indicating that groups were well-matched on age, weight, sex, and BMI. Table 1 shows the respiratory measures at each AIH stage (e.g., Post 30 min). No statistical difference between the cohorts was found for FVC. A main effect between ALS and the control cohort was observed for the MIP baseline ($p = 0.04$). However, no main effect between ALS and the control cohort was observed for MIP for the AIH treatment post 60 minutes. Additionally, no significant time effects (baseline, post 30, and post 60) were found for MIP. Thus, isometric maximal-effort volitional tests were not able to capture the effect of AIH. During each $P_{0.1}$ assessment, $ETCO_2$ was within normal limits for each group and fluctuated little over the course of each $P_{0.1}$ test. No significant group or time effects were found for $ETCO_2$, suggesting that AIH did not impact ventilation.

Table 3 describes the respiratory features extracted from the pressure data of the 15 subjects. Table 4 indicates significant results from the mixed-effects model analysis. An evident main effect of Time After Occlusion (100 ms, 200 ms, and 300 ms) can be seen across the three times points due to the pressure changing drastically as it transitions from AOR to DOR. No main effect of Cohort (ALS and control) or AIH stage (baseline, 30 min post, and 60 min post) is observed alone. This result indicates that not considering the different occlusion times fails to discriminate between the two groups. The two-way interaction between cohorts and AIH Stage is significant for the slope feature, with a slight increase of the slope for the control group after the AIH. In contrast there is a decrease in slope for the ALS group. In two cases, the P/MIP feature was the only variable to show significance in 1) a two-way interaction between Cohort and Time After Occlusion; 2) and a two-way interaction for AIH Stage and Time After Occlusion. P/MIP was also more significant than pressure or MIP alone, demonstrating that relating these two variables provides even more insights than analyzing them individually. These results confirm that the shape of the pressure waveform is impacted by both ALS and AIH. The three-way interaction for Cohort, AIH Stage, and Time After Occlusion was deemed significant for the pressure and P/MIP ratio features. While we see a constant negative increase in pressure for the control group as time after AIH advances, the ALS cohort presents a negative decrease 30 min post-AIH and an increase 60 min post-AIH. This observed increase at 60 min after AIH is compatible with the time required to increase synthesis of brain-derived neurotrophic factor (BDNF), necessary to cause phrenic long-term facilitation Baker-Herman et al. (2003).

We demonstrate weak but significant Spearman's correlation between age of ALS patients and $\Delta P_{0.3}$ ($\rho = 0.24$, $p = 0.04$), $\Delta S_{P_{0.1}}$ ($\rho = 0.30$, $p = 0.01$), $\Delta S_{P_{0.3}}$ ($\rho = 0.26$, $p = 0.03$) and $\Delta P_{0.3}$/MIP ($\rho = 0.24$, $p = 0.04$). However, when pre-AIH metrics and post-AIH metrics were analyzed separately, we see a correlation between age and $P_{0.3}$ ($\rho = -0.33$, $p = 0.003$), $S_{P_{0.1}}$ ($\rho = -0.24$, $p = 0.03$), $S_{P_{0.3}}$ ($\rho = -0.35$, $p = 0.001$) before AIH, but no correlation is significant after AIH. The one-way ANOVA analysis showed no significant statistical difference between changes in respiratory metrics of patients with limb ALS onset versus patients with bulbar ALS onset. The only respiratory metric to show statistical difference between patients with limb ALS onset versus control were $\Delta P_{0.3}$ and $\Delta S_{P_{0.3}}$ ($p = 0.02$ and $p = 0.002$). $\Delta P_{0.3}$ was also significantly different between patients with bulbar ALS onset versus control ($p = 0.02$). The Wilcoxon rank-sum test reported significantly different $\Delta P_{0.1}$/MIP and $\Delta P_{0.3}$/MIP between ALS patients who use NIV and those who do not. These results have to be interpreted with caution due to the small sample size.

Table 2
Standard Respiratory Measurements. Data are expressed as mean ± standard deviation.

Control			
	Baseline	Post 30	Post 60
FVC(%)	102.7 % ± 16.2 %	—	—
ETCO $_2$	39.3 ± 5.6	40.6 ± 5.0	40.1 ± 6.2
MIP	105.1 ± 23.2	—	102.0 ± 29.5
ALS			
	Baseline	Post 30	Post 60
FVC(%)	88.6 % ± 15.3	—	—
ETCO $_2$	41.3 ± 2.7	41.3 ± 2.7	41.0 ± 2.6
MIP	73.4 ± 24.7	—	70.6 ± 23.89

4. Discussion

The three primary findings from this study demonstrate that: 1) ALS patients have a higher negative pressure response to the occlusion than the control cohort at 300 ms; 2) AOR did not significantly change following AIH; 3) DOR of ALS patients significantly increased after AIH. These findings suggest the presence of both a preserved central respiratory drive and a robust compensatory pressure-generating capacity in this cohort of early-stage ALS patients. The results also support the expected outcome for AIH, which is not an increase in central drive but an increase in the DOR. Therefore, AIH is not driving the patient's respiratory system harder indiscriminately but providing additional respiratory capacity when a respiratory requirement is detected. Thus, the data suggest AIH allows patients to regulate and provide the necessary neuroplasticity to adjust their respiratory capacity. This personalized regulation prevents chronic elevations of central respiratory drive and avoids glutamate excitotoxicity, a potential contributor to motor neuron death in ALS, leading to further respiratory decompensation Seven and Mitchell (2019). This would be analogous to having a car engine continuously revving at its maximum revolutions per minute (RPMs), leading to the engine eventually overheating and failing, as well as the inability to regulate the car's ability to overcome new challenges (e.g., speeding up to pass another car). Proving patients with the ability to overcome these challenges, represented here by an unexpected respiratory load, might be critical to help with common issues that ALS patients go through, such as airway clearance and meeting new physical demands.

The analysis of the DOR demonstrates the importance of assessing pressure at later time points to understand the effects of AIH in both cohorts. AIH did not show any significant impact on the subjects until the pressure of an occlusion sustained for, at least, 200 ms was analyzed. Therefore, although the AOR of the subject is a great surrogate measurement for the respiratory drive, the DOR measurement is imperative to capture intrinsic attributes of the inspiratory pressure waveform. Capturing the response to an unexpected respiratory load, such as an occlusion, might also be more efficient in assessing respiratory function and how compromised patients can meet new respiratory challenges. Conversely to DOR, tidal volume and maximal-effort volitional tests such as sniff nasal inspiratory pressure (SNIP), MIP, or FVC are affected

Table 3
Respiratory features extracted from $P_{0.1}$ trials. Data are expressed as mean ± standard deviation.

Respiratory Features	Control			ALS		
	Baseline	Post 30	Post 60	Baseline	Post 30	Post 60
$P_{0.1}$	− 0.591±0.407	− 0.586±0.459	− 0.430±0.520	− 0.827±0.582	− 0.748±0.519	− 0.893±0.750
$P_{0.2}$	− 1.932±0.871	− 1.765±0.861	− 1.513±1.143	− 2.274±1.135	− 2.082±1.066	− 2.546±1.490
$P_{0.3}$	− 3.244±1.170	− 2.984±1.110	− 2.618±1.543	− 3.730±1.690	− 3.499±1.573	− 4.073±2.099
$S_{P_{0.1}}$	− 8.709±4.598	− 8.004±5.310	− 7.030±6.252	− 11.235± 6.658	− 10.208± 6.521	− 12.574± 7.949
$S_{P_{0.2}}$	− 13.559±5.882	− 11.933± 4.961	− 10.969± 7.263	− 14.607± 6.794	− 13.248± 6.606	− 16.698± 8.355
$S_{P_{0.3}}$	− 13.134±4.848	− 12.201± 4.174	− 11.097± 5.716	− 14.616± 7.529	− 14.201± 6.966	− 15.348± 7.835
$P_{0.1}$/MIP	− 0.006±0.004	− 0.006±0.005	− 0.004±0.005	− 0.013±0.012	− 0.011±0.009	− 0.015±0.016
$P_{0.2}$/MIP	− 0.019±0.010	− 0.018±0.010	− 0.015±0.012	− 0.034±0.023	− 0.031±0.020	− 0.043±0.034
$P_{0.3}$/MIP	− 0.032±0.013	− 0.029±0.012	− 0.026±0.016	− 0.055±0.032	− 0.052±0.031	− 0.068±0.049

Table 4
Significance p-values obtained using a Mixed-Effects Model for Repeated Measures at Baseline, Post 30 and Post 60 for each respiratory feature.

	Pressure	Slope	P/MIP
Time After Occlusion	< 0.001[a]	< 0.001[a]	< 0.001[a]
Cohort × AIH Stage	0.317	0.019[a]	0.507
Cohort × Time After Occlusion	0.317	0.450	< 0.001[a]
AIH Stage × Time After Occlusion	0.233	0.637	0.006[a]
Cohort × AIH Stage × Time After Occlusion	0.036[a]	0.899	0.011[a]

[a] $p < 0.05$

by sedation and certain medications Olsen et al. (2021), as well as effort, learning, and cognitive involvement. Assessing respiratory function with occlusions at quiet breathing removes these variables, putting subjects affected or not by disease at the same comparison level. Therefore, when we extend our analysis to the DOR, we are able to capture both the unconscious response and the subject's individual response to the load. Future work may explore other methods to characterize the AOR and DOR, such as frequency decomposition Napoli et al. (2022).

Our $P_{0.1}$ findings are in alignment with those of previous studies that used $P_{0.1}$ to measure respiratory drive in ALS patients and characterize breathing dynamics (Vitacca et al., 1997; Pinto et al., 2021. Vitacca and coworkers Vitacca et al. (1997) measured $P_{0.1}$ from non-occluded breaths using an esophageal catheter, and they noted a preserved drive in ALS patients, even among those with progressive respiratory weakness. Pinto and coworkers Pinto et al. (2021) measured $P_{0.1}$ in a large cohort of adults living with ALS during their first clinical visit. $P_{0.1}$ was expressed as both a percentage of reference $P_{0.1}$ value and a percentage of MIP. Many patients exhibited a high central drive, but wide variability in $P_{0.1}$ was noted among those approaching respiratory failure. While occlusions are conventionally applied and are undetected during quiet breathing, Pinto and coworkers measured $P_{0.1}$ while instructing patients to complete an inspiratory effort against a known occlusion Pinto et al. (2021). Thus, it is uncertain if fluctuations in $P_{0.1}$ reflected fundamental differences in clinical phenotype, a metabolic compensation, or alternatively, if the variability could be attributed to differences in subjects' voluntary/conscious pressure generation. Our findings suggest that, when occlusion was presented as an undetected stimulus, larger group differences occurred later in the inspiration effort ($P_{0.2}$, $P_{0.3}$) as DOR's. These time points are typically attributed to voluntary compensatory efforts Whitelaw et al. (1975).

In regards to the relationship between patients' age and inspiratory effort ($P_{0.3}$, $S_{P_{0.1}}$ and $S_{P_{0.1}}$), we suspect that the change in correlation, from significant before AIH to non-significant after AIH, may be due to several factors. One relationship that the data clearly identified was that not everyone responded similarly to the AIH perturbation. This could be because some people responded more favorably (e.g., super-responders) than others. Therefore, this may create a subset of patients who benefit more from AIH protocols than others. Future studies should examine this more closely in a healthy population, at first, to determine the significance of this relationship beyond a subset of individuals. Furthermore, AIH seemed to have a higher impact on older ALS patients than on younger patients, thus leading to almost 0 correlation after AIH. While there is no research on humans yet, a study on rodent models has shown that pLTF is enhanced in end-stage $SOD1^{G93A}$ rats (Nichols et al., 2013, 2015). Further examination in a larger cohort is required to verify this hypothesis.

With respect to AIH and its effect in $P_{0.1}$, Sutor and colleagues Sutor et al. (2021) measured $P_{0.1}$ responses to a single trial of AIH in stable adults with chronic spinal cord injury. Similar to our results, they found baseline $P_{0.1}$ remained normal and did not change following a single session of AIH. However, MIP significantly increased after AIH, suggesting that AIH-induced gains in voluntary function may occur independently of an increased respiratory drive. Initial pilot testing of AIH in ALS revealed significant increases in resting breathing, including tidal volume, minute ventilation, and respiratory muscle EMG amplitude Sajjadi et al. (2022). Since AIH increased later inspiratory pressure generation in ALS without impacting $P_{0.1}$, we speculate that AIH-triggered synaptic plasticity augmented patient's compensatory respiratory muscle recruitment in response to occlusions without significantly altering the underlying drive. We note a larger variance observed within the ALS group, which could be due to the heterogeneous nature of the disease. Alternatively, it may represent distinct compensatory muscle recruitment patterns facilitated by AIH. These issues require further study in a larger cohort with approaches that can capture statistical biases from missing or unaccounted information Napoli et al. (2017).

4.1. Strengths and limitations of the study

Resting breathing and occlusion pressure response measurements were conducted using a silicone facemask to overcome potential air leaks due to bulbar involvement of patients. The $P_{0.1}$ responses for patients and controls appear lower than values reported elsewhere Baydur (1991), but this could be attributed to the compliance of the mask, as compared to a mouthpiece. However, the use of a mask enables $P_{0.1}$ measurement while minimally affecting the patient's swallowing ability. Thus, allowing the pressure measurement to be consistent and precise cross recorded subjects.

A strength of the study is the impact of the dose response of the AIH had on the subject. Strictly utilizing a single therapeutic session of AIH, we demonstrated a substantial increase in respiratory capacity for ALS compromised patients to meet new challenging respiratory demands. In addition subjects demonstrated that the AIH therapeutic session were well-tolerated and safe for a compromised patient population. This is crucial to document the tolerance of procedures when studying critically ill patient populations, which as been also previously reported in existing literature. Due to the tolerance and positive impact of AIH, future studies should include larger subject cohorts to explore various

protocols of AIH delivery (i.e., number AIH sessions, durations,etc.) to the optimally impact prolonged respiratory benefits.

4.2. Clinical significance

Clinical maximal volitional respiratory function tests used to monitor ALS patients may be insufficient at measuring respiratory drive. The current study outlined how measuring inspiratory occlusions at three different time points may be a more sensitive measure at detecting central respiratory drive within neuromuscular diseased patient populations. Such measurements could provide insights to practitioners and offer better management of respiratory symptoms with earlier detection of ventilatory failure. AORs and DORs were utilized to better characterize respiratory drive and pressure-generated waveforms pre-and post-AIH administration. Following a single session of AIH, DORs measured 60 min post-AIH demonstrated a significant change in respiratory outcome. The AOR and DOR analysis indicate a potential increase in pressure-generating capability without a large increase in central respiratory drive, despite increased minute ventilation for ALS patients. This also supports AIH as a safe treatment for ALS. This is due to not continuously driving the patient's respiratory drive higher unnecessarily but providing additional pressure when a load is detected and must be overcome. This additional support for inspiratory drive, when necessary, can help prolong unassisted breathing for ALS patients without causing fatigue. This potential gain can help improve quality of life and clinical measurements (e.g., ALSFRS-R). Although these changes were observed following a single session of AIH, additional sessions are necessary to further evaluate the clinical utility and maintenance of therapeutic benefits.

5. Conclusion

This study investigated subjects' inspiratory pressure response to unexpected airway occlusion. We demonstrate that going beyond the 100 ms time point after an occlusion provides valuable information to better understand the effects of AIH in ALS patients versus their control counterparts. Along with the new time points, we also demonstrate that the metrics proposed in the paper allow us to characterize changes in the shape of the occlusion pressure wave. While AIH did not significantly impact the underlying respiratory drive or the MIP of patients or controls, AIH elicited apparent facilitation of voluntary compensatory pressure generation in those with ALS. This effect, however, is only seen when extending the pressure analysis to 200 ms and 300 ms post-occlusion. These findings are consistent with a potential role for AIH as a means to enhance respiratory motor function in earlier stages of ALS without excessively increasing respiratory drive. Due to the heterogeneous nature of ALS and lack of neuro-respiratory prognostics tools, follow-up experiments and analyzes are needed. New approaches are needed to improve the identification and distinctions in the pressure-generating capacity of patients, functional flow dynamics, and optimal AIH protocols that would generate the best respiratory motor facilitation for neuromuscular diseased patient populations whose breathing becomes compromised.

Acknowledgements

Research reported in this publication was supported by the University of Florida Moonshot Initiative and the University of Florida Clinical and Translational Science Institute, which is supported in part by the NIH National Center for Advancing Translational Sciences under award number UL1TR001427. We would also like to knowledge the support from the NIH National Heart, Lung, and Blood Institute through their LRP award number L30HL159818. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

References

Baker-Herman, T.L., Fuller, D.D., Bavis, R.W., Zabka, A.G., Golder, F.J., Doperalski, N.J., Johnson, R.A., Watters, J.J., Mitchell, G.S., 2003. BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. Nat. Neurosci. 7 (1), 48–55.

Baydur, A., 1991. Respiratory muscle strength and control of ventilation in patients with neuromuscular disease. Chest 99 (2), 330–338.

Bourke, S., Shaw, P., Gibson, G., 2001. Respiratory function vs sleep-disordered breathing as predictors of QOL in ALS. Neurology 57 (11), 2040–2044.

Bourke, S.C., Bullock, R.E., Williams, T.L., Shaw, P.J., Gibson, G.J., 2003. Noninvasive ventilation in ALS: indications and effect on quality of life. Neurology 61 (2), 171–177.

Cheah, B.C., Boland, R.A., Brodaty, N.E., Zoing, M.C., Jeffery, S.E., McKenzie, D.K., Kiernan, M.C., 2009. INSPIRATIonAL – INSPIRAtory muscle training in amyotrophic lateral sclerosis. myotroph. Lateral Scler. 10 (5–6), 384–392.

Faraway, J., 2016. Extending the Linear Model with R: Generalized Linear, Mixed Effects and Nonparametric Regression Models. CRC Press Taylor & Francis Group,, Boca Raton.

Golub, G., 1965. Numerical methods for solving linear least squares problems. umer. Math. 7 (3), 206–216.

Iżecka, J., Stelmasiak, Z., Balicka, G., 2003. Respiratory function in amyotrophic lateral sclerosis. Neurol. Sci. 24 (4), 288–289.

Kiernan, M.C., Vucic, S., Cheah, B.C., Turner, M.R., Eisen, A., Hardiman, O., Burrell, J.R., Zoing, M.C., 2011. Amyotrophic lateral sclerosis. lancet 377 (9769), 942–955.

Lahrmann, H., Wild, M., Zdrahal, F., Grisold, W., 2003. Expiratory muscle weakness and assisted cough in ALS. myotroph. Lateral Scler. Other Mot. Neuron Disord. 4 (1), 49–51.

Lange, D.J., Lechtzin, N., Davey, C., David, W., Heiman-Patterson, T., Gelinas, D., Becker, B., Mitsumoto, H.M., The HFCWO Study Group, 2006. chest wall oscillation in ALS: An exploratory randomized, controlled trial. Neurology 67 (6), 991–997.

Laveneziana, P., Albuquerque, A., Aliverti, A., Babb, T., Barreiro, E., Dres, M., Dubé, B.-P., Fauroux, B., Gea, J., Guenette, J.A., Hudson, A.L., Kabitz, H.-J., Laghi, F., Langer, D., Luo, Y.-M., Neder, J.A., O'Donnell, D., Polkey, M.I., Rabinovich, R.A., Rossi, A., Series, F., Similowski, T., Spengler, C., Vogiatzis, I., Verges, S., 2019. ERS statement on respiratory muscle testing at rest and during exercise. Eur. Respir. J. 53 (6), 1801214.

Lechtzin, N., Rothstein, J., Clawson, L., Diette, G.B., Wiener, C.M., 2002. Amyotrophic lateral sclerosis: evaluation and treatment of respiratory impairment. Amyotroph. Lateral Scler. Other Mot. Neuron Disord. 3 (1), 5–13.

N.J. Napoli, M.E. Kotoriy, W. Barnhardt, J.S. Young, L.E. Barnes, Addressing bias from non-random missing attributes in health data.IEEE EMBS Intern. Conf. on Biomedical and Health Informatics, 265–268, 2017.

Napoli, N.J., Rodrigues, V.R., Davenport, P.W., 2022. Characterizing and modeling breathing dynamics: Flow rate, rhythm, period, and frequency. Front. Physiol. 12.

Nichols, N.L., Gowing, G., Satriotomo, I., Nashold, L.J., Dale, E.A., Suzuki, M., Avalos, P., Mulcrone, P.L., McHugh, J., Svendsen, C.N., et al., 2013. Intermittent hypoxia and stem cell implants preserve breathing capacity in a rodent model of amyotrophic lateral sclerosis. Am. J. Respir. Crit. Care Med. 187 (5), 535–542.

Nichols, N.L., Satriotomo, I., Harrigan, D.J., Mitchell, G.S., 2015. Acute intermittent hypoxia induced phrenic long-term facilitation despite increased sod1 expression in a rat model of als. Exp. Neurol. 273, 138–150.

Olsen, W., Morris, K., Bolser, D., 2021. Proposed mechanisms of opioid-induced respiratory suppression mediated by actions at excitatory synapses within the inspiratory network: Results from a neuromechanical computational model of the respiratory control system. FASEB J. 35 (S1).

Pinto, S., Swash, M., Carvalho, M.De, 2021. Mouth occlusion pressure at 100ms (p0. 1) as a respiratory biomarker in amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. Front. Degener. 22 (1–2), 53–60.

Sajjadi, E., Seven, Y.B., Ehrbar, J.G., Wymer, J.P., Mitchell, G.S., Smith, B.K., 2022. Acute intermittent hypoxia and respiratory muscle recruitment in people with amyotrophic lateral sclerosis: a preliminary study. Exp. Neurol. 347, 113890.

Seven, Y.B., Mitchell, G.S., 2019. Mechanisms of compensatory plasticity for respiratory motor neuron death. Respir. Physiol. Neurobiol. 265, 32–39.

Seven, Y.B., Nichols, N.L., Kelly, M.N., Hobson, O.R., Satriotomo, I., Mitchell, G.S., 2018. Compensatory plasticity in diaphragm and intercostal muscle utilization in a rat model of ALS. Exp. Neurol. 299, 148–156.

Singh, D., Verma, R., Garg, R.K., Singh, M.K., Shukla, R., Verma, S., 2011. Assessment of respiratory functions by spirometry and phrenic nerve studies in patients of amyotrophic lateral sclerosis. J. Neurol. Sci. 306 (1–2), 76–81.

Sutor, T., Cavka, K., Vose, A.K., Welch, J.F., Davenport, P., Fuller, D.D., Mitchell, G.S., Fox, E.J., 2021. Single-session effects of acute intermittent hypoxia on breathing function after human spinal cord injury. Exp. Neurol., 113735

Vitacca, M., Clini, E., Facchetti, D., Pagani, M., Poloni, M., Porta, R., Ambrosino, N., 1997. Breathing pattern and respiratory mechanics in patients with amyotrophic lateral sclerosis. Eur. Respir. J. 10 (7), 1614–1621.

W.A. Whitelaw and J.P. Derenne, Airway occlusion pressure.74(4), 1475–1483, Apr. 1993.

Whitelaw, W.A., Derenne, J.-P., Milic-Emili, J., 1975. Occlusion pressure as a measure of respiratory center output cm conscious man. Respir. Physiol. 23 (2), 181–199.

Zinman, L., Cudkowicz, M., 2011. Emerging targets and treatments in amyotrophic lateral sclerosis. Lancet Neurol. 10 (5), 481–490.