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RESPIRATORY CAUSES IMPACTING PILOT PERFORMANCE

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14. ABSTRACT
This report describes the research into the cardiovascular, respiratory, and cognitive impacts of "operationally relevant" environments including oscillating O_2/N_2 concentrations in the background of mild altitude; increased inspiratory and expiratory threshold loaded breathing; and positive-pressure breathing under elastic chest wall loading. The findings of this work suggest these operationally relevant stressors may cause acute, albeit mild decrements in cardiorespiratory physiology and cognitive performance. As such, the current data demonstrates minimizing these respiratory perturbations during flight may prove useful in reducing deleterious cardiorespiratory events and preserving attentional performance.

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TASK 1: VARIABLE OXYGEN CONCENTRATION STUDY

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1.0 SUMMARY

There are growing concerns over the unexpected physiological events among pilots of US Air Force and US Navy high-performance aircraft. Recent investigations dealing with advanced aircrew flight equipment in high-G capable aircraft have revealed a number of significant challenges linked to the respiratory system that may play a role in the occurrence of unexplained physiological events (UPEs). Significant lung related stressors include high levels of inspired oxygen along with variable and rapid changes in oxygen tension along with dry air. These stressors may predispose pilots to airway inflammation, lung injury and potentially contribute to the constellation of unexplained physiological events (UPEs) in aircrew of high-performance aircraft. Repetitive exposures may further increase risks to the respiratory system and evolving research also suggests a further link between lung function, gas exchange, inflammation, and cognitive performance. Effective aircrew operations are dependent upon optimal physiologic performance of operators under adverse environments. Based on preliminary findings from USAFSAM, rapid variability in inspired oxygen concentration over windows of time, in a background of mild-moderate altitude may alter normal concentrations of alveolar nitrogen resulting in heterogeneous atelectasis, mild ventilation–perfusion abnormalities (VA/Qc) and when sustained for over 45 minutes, contribute to the "symptoms" observed in pilots. During flight, there are functionally varying inspired oxygen concentrations delivered to the pilots due to fluctuations in the on-board oxygen delivery systems and environmental pressure. There appears to be a combination of exposure variables, e.g., oscillations in the balance of inspired oxygen/nitrogen ratios, how often the transitions occur and the duration these exposures are experienced before mild changes in lung function are noted. Suggesting that the toxicity of hyperoxia and oxygen therapy to the lungs and brain is not limited to long exposures most often seen in the insensitive care setting [1-4]. The

purpose of this study was to determine whether "operationally-relevant" environmental challenges may cause acute decrements in lung and cerebral function.

Thirty, healthy participants (Age: 29 ± 5 yr) were recruited for this study and assigned to one of three study arms: 30s, 60s, or 120s oscillations of 80/20 and 30/70 O_2/N_2 inspired gas ratios. Participants visited the laboratory on 2 separate occasions. During *Visit 1*, participants were assessed for height, weight, blood pressure, 12 lead ECG, basic pulmonary function testing, and CBC to rule out anemia. Participants were then familiarized with the Masked Conjunctive Continuous Performance Task, Rapid Cognitive Assessment Task, and the Well Living Lab Cognitive Test. For *Visit 2*, participants reported the Mayo Clinic Hypobaric Chamber. During this visit, a lung ultrasound was taken. Following this, a Respiratory Therapist placed an arterial catheter in the radial artery. The participant was then instrumented for monitoring purposes with ECG, thoracic impedance, SpO2, near-infrared spectroscopy, and cerebral blood flow (CBF). This was followed by baseline testing consisting of fractional exhaled NO (F_ENO); forced oscillation of the airways; blood draws for arterial blood gases (ABG); a rebreathe method for pulmonary blood flow (Vc), alveolar – capillary conductance (Dm), and lung clearance index (LCI); and spirometry. After baseline testing, the chamber was brought to an altitude of 8,000ft. Participants were placed on a mouthpiece and were instructed to spontaneously breath while being exposed to the oscillating gas concentrations for 45 min. Cerebral and peripheral O_2 saturation, impedance, ECG, and transcranial doppler (TCD) were monitored continuously and every 8 minutes symptoms were assessed. Additionally, every 8 minutes, the participants were instructed to complete a round of cognitive testing consisting of playing each cognitive test once. After 45 minutes of exposure, baseline measures were repeated at sea level. This was followed by another 45-minute exposure, after which repeat baseline measures were obtained, again at sea level. Approximately 45 minutes

after the end of the second exposure, baseline measures were repeated.

Repeated measures analyses of variance (ANOVAs) were used to determine the effect of exposure to oscillating O_2/N_2 concentrations in the background of mild altitude on respiratory mechanics, gas exchange, and hemodynamics. Data obtained during the chamber visit were analyzed using separate repeated measures ANOVAs. While we observed no significant changes in subjects' symptoms ratings in response to any oscillation rate nor was there evidence of airway inflammation as measured by F_ENO. Despite this, we did observe alterations in respiratory mechanics in response to oscillating O_2/N_2 concentrations. There was evidence of decreased peripheral airway reactance in the shorter oscillations, but not the longer oscillations (38.6% vs. 6.5% and 3.8% decrease, for 30s, 60s, and 120s oscillations respectively). Another interesting finding was the reduced operational lung volumes, as measured by forced vital capacity and expiratory lung volumes, and expiratory flow rates observed ($P < 0.05$). Additionally, our data demonstrated no effect of oscillating O_2/N_2 concentrations on alveolar-capillary membrane conductance. However, we did observe a systemic decline in Q and an associated reduction in Vc ($P < 0.05$). The maintenance of Dm and concomitant reduction in Vc resulted in an increased Dm/Vc (19.5%, 11.6%, and 26.5% for 30s, 60s, and 120s oscillations respectively) . Furthermore, there was an observed a systematic reduction in LCI across all exposure types with a return towards baseline at the recovery testing period. Taken together, these data suggest exposure to oscillating O_2/N_2 concentrations may engender very mild alveolar atelectasis with no attendant influence on the lungs ability to diffuse gases to the blood.

Generalized additive mixed effects models (GAMMs) were used to determine the impact of the duration of the exposure on respiratory mechanics, gas exchange, hemodynamics, and cognitive performance. We observed no change in minute ventilation (VE), respiratory rate (RR), or tidal

volume (Vt) in response to the 30s oscillation exposure. Similarly, there were no changes in VE during the 120s oscillations. However, our data did demonstrate a significant decrease in RR ($P < 0.05$) and a compensatory, non-significant increase in Vt ($P = 0.06$). We observed a similar trend in respiratory patterns during the 60s oscillations insofar as RR was dampened and Vt was increased. However, in contrast to the 120s oscillation exposures, VE did decrease during the 60s oscillations. Additionally, our data demonstrated an increase in HR within each study arm for all exposure types. Furthermore, we observed an increase in BP during the 30s and 120s oscillations, but no change in the 60s oscillation cohort. Our data also demonstrated systematic increases in cerebral oxygenation (RSO_2). Taken together, these data suggest exposure to hyperoxic hypobaria may have little effect on respiratory patterns but that longer exposures to oscillating hyperoxia may exert a somewhat more pronounced effect on respiratory patterns. Further, these data suggest a delicate balance between central and peripheral chemoreceptor and sympathetic activity that may be influenced by the length of hyperoxia and/or hypocapnia oscillating exposures in the background of mild altitude.

The findings of this work suggest exposures to shorter oscillations in O_2/N_2 concentrations may present the strongest effect on respiratory mechanics whereas longer oscillations appear to have a more pronounced influence on pulmonary function while at mild altitude, but in context, the overall influence or challenge to the respiratory system appears relatively mild. We propose two primary mechanisms for these findings. First, shorter exposures to relative normoxia may not have been sufficient time to recover N_2 gas tensions, not allowing to a return to baseline PO_2. As such. shorter oscillations rates may in fact expose participants to longer times with elevated PO_2 which may have a compounding influence wherein consistently elevated PO_2 may induce more decrements to respiratory function. Second, we reason the combination of hyperoxia and altitude

exposure may alter the relationship between the peripheral and central chemoreceptors and downstream sympathetic drive. As such, the shorter oscillation exposures may further increase sympathetic drive, causing more negative cardiovascular impacts. Conversely, our data do not demonstrate any significant alterations in cognitive function, either in reaction time or accuracy, in response to the differing exposure.

2.0 INTROUCTION

Recent investigations dealing with advanced aircrew flight equipment in high-G capable aircraft have revealed a number of significant challenges linked to the respiratory system that may be responsible for the occurrence of unexplained physiological events (UPEs). Significant lung related stressors include the high elastic and resistive loads to breathing, thoracic compression, high levels of inspired O_2 along with variable and rapid changes in O_2 tension and dry air often in the setting of Valsalva and strain maneuvers resulting in pressure differentials across the lungs. The high work and cost of breathing may challenge cognitive skills, and in conjunction with other stressors may predispose pilots to airway inflammation, lung injury and potentially contribute to the constellation of UPEs in aircrew of high-performance aircraft. Repetitive exposures may further increase risks to the respiratory system and evolving research also suggests a further link between lung function, gas exchange, inflammation, and cognitive performance. Effective aircrew operations are dependent upon optimal physiologic performance of operators under adverse environments. Currently there is no clear understanding of the primary environmental stressors of pilots on lung physiology or how challenges to the respiratory system impact cognitive performance.

As noted above, a significant contributor to the development of UPEs is believed to be high levels of inspired O_2 along with rapid oscillations in O_2 tension. These rapid oscillations of high O_2 concentrations may result in lung injury, similar to ventilator-induced lung injury (VILI). There are four classical mechanisms of VILI: atelectrauma, barotrauma, volutrauma, and biotrauma. However, the mechanisms most pertinent to the perturbations imposed upon pilots, and highlighted below, are atelectrauma and biotrauma.

Atelectrauma:

High inspired O_2 concentrations are associated with increased reactive oxygen species (ROS), most notably superoxide and hydroxyl radicals, and may contribute to surfactant depletion [5]. More specifically, surfactant exposed to high O_2 concentrations (i.e. 100%) had a significantly reduced diameter compared with surfactant exposed to normal O_2 concentrations [6]. Surfactant plays an integral role in reducing and normalizing surface tension in alveoli of varying diameters. As such, depletion of surfactant may subject the alveoli to collapsing upon expiration (i.e. atelectasis). In addition to surfactant depletion, high inspired O_2 may also result in absorption atelectasis [7]. Oxygen is highly soluble and diffuses rapidly into the pulmonary vasculature, and in the absence of sufficient nitrogen concentrations, the resulting partial pressure is insufficient to maintain alveoli patency. A reduced patency would subject the alveoli to a greater likelihood of atelectasis. Not only may high O_2 concentration contribute to atelectrauma, but the oscillating in partial pressure of oxygen (PO_2) may play a functional role as well, suggesting that the toxicity of hyperoxia and oxygen therapy to the lungs and brain is not limited to long exposures [1-4].

The oscillations in PO_2 originate in the lungs in the presence of within-breath recruitment/derecruitment of alveoli [8]. This cyclic alveolar recruitment/derecruitment may result

in surfactant depletion and is an important mechanism of lung injury [9, 10]. In fact, the proposed mechanism for these oscillations is the recruitment of atelectasis and the resulting pulmonary shunt fractions [11]. As such, the breath-by-breath collapse of dependent lung regions leads to large alveolar PO_2 oscillations with the varying shunt ratios throughout the respiratory cycle. Surfactant depletion and the resultant dysregulation of alveolar surface tension coupled with an increased rate of alveolar recruitment/derecruitment may increase the likelihood of alveolar collapse. In fact, oscillating PO_2 resulted in significantly higher lung injury scores in vivo [8]. These alveolar PO_2 oscillations are transmitted to arterial oxygen tension (PaO_2) and in turn, alveolar recruitment/derecruitment patterns can be quantified by changes in PaO_2 [9, 12].

Biotrauma:

Mechanical lung injury may also trigger an extensive biological response, including activation of a proinflammatory and pro-injurious cytokine cascade termed biotrauma. [13]. Alveolar overdistention and recruitment/derecruitment can induce a proinflammatory cytokine cascade through three distinct pathways: (i) neutrophil infiltration [14]; (ii) increased pulmonary cytokine concentrations [15]; and (iii) increased circulating cytokine levels [16, 17]. Furthermore, high tidal volume ventilation may contribute to systemic inflammation by translocating bacteria from the airspace into the pulmonary circulation [18]. Consequently, injurious ventilation may result in a 50-fold increase in proinflammatory cytokine concentration in broncho-alveolar lavage fluid [19, 20]. There is an extensive interaction between the pulmonary system and peripheral organ systems, wherein the entire volume of blood passes through the pulmonary circulation every minute. The passive diffusion mechanism, derived from unbalanced oxygen and carbon dioxide gradients, allows for diffusion of gases from the blood to breath across the pulmonary-capillary membrane. Consequently, this mechanism also allows for the diffusion of molecules produced systemically

into the blood. Therefore proinflammatory and pro-injurious cytokines produced in the pulmonary tissue have a readily available interface through which they can affect uninvolved, systemic organs [21].

Therefore, the purpose of the current proposal is to determine whether other "operationally-relevant" environmental challenges may cause acute or chronic decrements in lung health. A combination of variable respiratory loads, pressure changes across the lungs, altered blood flow and volumes in the thorax and head, high oxygen levels, dry air all challenge the normal adaptive abilities of the lungs. Inflammation, altered fluid regulation, congestion, smooth muscle dysregulation have all been shown to occur with these stressors under challenging environments or in clinical conditions, however the threshold for when these cause maladaptive changes in the lungs of pilots is unclear and the combination of challenges may be particularly difficult to maintain lung health.

3.0 METHODS, ASSUMPTIONS, AND PROCEDURES

3.1 Participants

Thirty healthy participants (Age: 29 ± 5 yr) were recruited for this study. Ten participants (n=8 males, n=2 females for 30s and 120s oscillations, and n=7 males, n=3 females for 60s oscillations) were assigned to one of the three exposure limbs in an attempt to achieve a representative sample of the Air Force pilot population. Participants had no known history of cardiac, pulmonary, and/or metabolic disease, and no reported mental or psychological disorders of attention. Each participant completed both exposures except for 1 participant who did not complete the second exposure (n=30 and n=29, for completion of both exposure and completion of 1 exposure respectively). The present study conformed to the principles outlined in the Declaration of Helsinki and was approved

by the Mayo Clinic Internal Review Board.

3.2 Experimental Design

To determine the impact of variable inspired O_2/N_2 ratios at different oscillation rates (i.e., 30s, 60s, and 120s) in the background of mild altitude (i.e., 8,000ft) participants visited the laboratory for a screening visit study visit on separate days. A description of the methods and procedures is provided below.

3.2.1 Screening Visit

During this visit, participants were assessed for height (Ht), weight (Wt), blood pressure (BP), 12 lead ECG, basic pulmonary function testing (PFT), and CBC to rule out anemia. Participants were then familiarized with the Masked Conjunctive Continuous Performance Task (MCCPT), Rapid Cognitive Assessment Task (RCAT), and the Well Living Lab Cognitive Test (WLL).

3.2.2 Cognitive Tests

3.2.2.1 Masked Conjunctive Continuous Performance Task

The masked conjunctive continuous performance task (MCCPT) is a complex choice reaction time (RT) task that requires participants to either respond or withhold a response to a visual stimulus. The visual stimulus consisted of a colored mask, comprised of four superimposed figures (circle, square, triangle, and hexagon) in different colors (red, blue, yellow, and green). To avoid habituation effects, minor movements (e.g., "jittering" of the image) in which every 10-20 ms two mask-images were alternated, one of which had thicker outlines for the superimposed figures. The mask appeared at the center of the screen and disappeared when it was replaced by either a target

or distractor shape for 100 ms. The mask then reappeared immediately, generating a pre- and post-masking of each target and distractor. The distractor shape was a red circle and target stimuli were either similar in shape (blue and yellow circle), similar in color (red hexagon and red triangle), or completely different (blue hexagon and yellow triangle). All target and distractor shapes appeared at the center of the screen with an inter-stimulus interval between 2,000 and 5,000 ms. The task was to press as fast as possible for all shapes and colors that were not a red circle. Subjects were instructed to do nothing when presented with a red circle. We developed a novel microcontroller-based device to implement the MCCPT – the device provided RT values with a sub-millisecond accuracy.

3.2.2.3 Rapid Cognitive Assessment Task

The rapid cognitive assessment task (RCAT) is a simple hand eye coordination test that required participants to rapidly click moving stimuli as they appeared on the screen in order to maximize a score used as an indicator of performance. The stimuli consisted of boxes that would appear, move semi-randomly around the screen for a short time (6 seconds), then disappear. Successfully clicking on a box increased the score and clicking elsewhere decreased the score. Box spawn rate was tied to the score, with a higher score resulting in a higher frequency of box spawns. The score would decay every 100 ms, at a rate that increased proportionally to the number of boxes on the screen. The combination of score-based box spawn rate and box-based score decay created a varying difficulty that adapted to each user's skill level. Every 8 seconds, a stationary yellow box would be presented in a semi-random location chosen to be in the subject's peripheral vision (using the cursor's location as an approximate indicator of focal position). Clicking a yellow box within 1300 ms after appearance would spawn a blue box (again in the subject's peripheral vision), with

a significantly increased score value as an incentive for the subject. If the yellow box was not successfully clicked, it would turn red and remain on screen for 4 seconds before disappearing, decrementing the player's score if clicked before it disappeared. The combination of yellow, red, and blue boxes served as an attention reset (to minimize fatigue) and provided limited reaction time measures. The subject was instructed to click the grey, yellow, and blue boxes as rapidly and accurately as possible.

3.2.2.3 Well Living Lab Cognitive Test

The well living lab cognitive test (WLL) is a reaction time test that requires participants to respond to a visual stimulus. The visual stimulus consisted of a single digit number from 1 to 9 (with 5 excluded). The stimulus was either yellow or purple in color. Each stimulus was presented in the middle of the screen and disappeared when the subject responded to it. Each subsequent stimulus appeared 500 ms after the previous one disappeared. Each test consisted of 48 presentations (3 of each stimuli) presented in random order. The task was to respond to the presentation by pressing one of two buttons, labelled "yes" and "no". For yellow stimuli, the subject was instructed to press "yes" if the number was greater than 5, and no if it was less than 5. For purple stimuli, they were instructed to press "yes" if the number was even, and "no" if the number was odd. We used the same novel microcontroller-based device that was used for the MCCPT.

3.2.3 Hypobaric Chamber Study Visit

Participants presented to the Mayo Clinic Hypobaric Chamber for the study visit. Before initiation of the altitude study visit, a hyperbaric RN provided a safety screening to include: (i) intake and documentation of vital signs; (ii) safety screening for prohibited materials; and (iii) safety screening for exposure to hyperbaric conditions within the last 24 hours. A lung ultrasound was

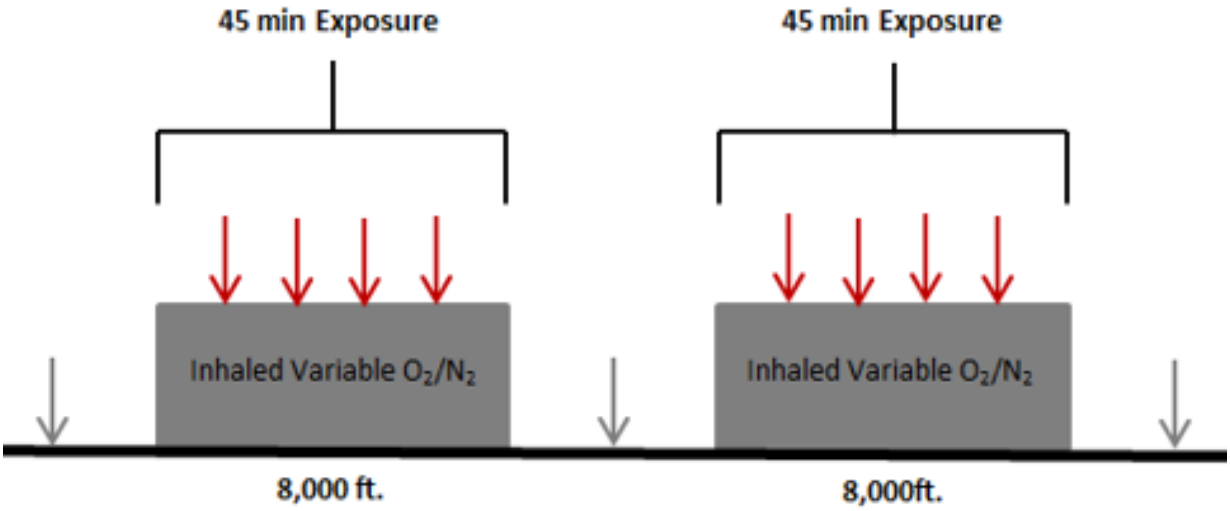
taken (Philips Lumify Ultrasound, Philips Healthcare Systems) utilizing 28 sonographic windows. Following this, a Respiratory Therapist placed an arterial catheter in the radial artery for the purpose of blood draws and continuous BP measurements. The participant was instrumented for monitoring purposes with ECG (GE Analytical Instruments), thoracic impedance (Model 2994 THRIM, UFI), SpO2 (Instrumentation Laboratory), near-infrared spectroscopy (NIRS) (SenSmart Model X-100, Nonin), and cerebral blood flow (CBF) via transcranial doppler (TCD) (Multigon Industries, Yonkers, NY) when possible.

Baseline testing consisted of fractional exhaled NO (F_ENO) (NObreath F_ENO Monitor, Bedfont Scientific Ltd.); forced oscillation of the airways (FOT) (Resmon Pro Forced Oscillation Technique, MGC Diagnostics); blood draws for arterial blood gases (ABGs); rebreathe for pulmonary blood flow, alveolar – capillary conductance, and lung clearance index (Marquette 110 Medical Gas Analyzer Mass Spectrometer, Perkins Elmer; Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments); and spirometry (MedGraphics Cardiorespiratory Diagnostic System, Medical Graphics Corporation).

After baseline testing, the chamber was brought to an altitude of 8,000ft. (International Standard Atmosphere ~565mmHg to account for variations in ambient barometric pressure) at a rate of 2,500ft/min. Participants were placed on a mouthpiece attached to Hans-Rudolph 4285 Series Switching Valve (Hans Rudolph) capable of switching between open-circuit breathing bags with the 80/20 and 30/70 O_2/N_2 ratios at the required frequency (30s, 60s, or 120s). Cerebral and peripheral O_2 saturation, impedance, ECG, and TCD were monitored continuously and every 8 minutes symptoms were assessed. Additionally, every 8 minutes, the participants were instructed to complete a round of cognitive testing consisting of playing each cognitive test once. After 45

minutes of exposure (henceforth referred to as Arm 1), baseline measures were repeated at sea level (i.e., midpoint). This was followed by another 45-minute exposure (henceforth referred to as Arm 2), after which repeat baseline measures were obtained, again at sea level (i.e., post). Approximately 45 minutes after the end of the second exposure, baseline measures were repeated (i.e., recovery). Participants were instructed to remain on the mouthpiece for the duration of the exposure.

Figure 1. Study design



↓
- Arterial line for blood gases
- Spirometry and FOT
- Rebreathe pulmonary blood flow, alveolar – capillary conductance, lung clearance index
- Lung ultrasound (fluid and lymph flow)

↓
- ECG
- Oxygen saturation
- Every 8-10 minutes dyspnea will be assessed

Variable oxygen concentration exposure for both exposures followed one of three protocols:
Protocol A (n=10) - 90 m exposure breathing [oscillating between 80 O_2/20 N_2 and 30 O_2/70 N_2 gas every 30 s]
Protocol B (n=10) - 90 m exposure breathing [oscillating between 80 O_2/20 N_2 and 30 O_2/70 N_2 gas every 60 s]
Protocol C (n=10) - 90 m exposure breathing [oscillating between 80 O_2/20 N_2 and 30 O_2/70 N_2 gas every 120 s]

3.3 Measured and Computed Variables

3.3.1 Respiratory Mechanics and Airway Inflammation

Fractional exhaled nitric oxide (F_ENO) was measure at end-expiration with a handheld device (NObreath F_ENO Monitor, Bedfont Scientific Ltd.) and is reported in ppb. Participants were instructed to take a deep breath and exhale at a constant flow rate until end-expiration on a collection straw attached to the NObreath F_ENO Monitor. Participants were provided visual feedback on appropriate flow and expiratory time to maintain consistency. Fractional exhaled nitric oxide (F_ENO) measures were taken at the baseline, midpoint, post, and recovery time periods.

Forced oscillation of the airways (FOT) were measured with the Resmon Pro Forced Oscillation Technique. This technique measures airway resistance (Rrs) and reactance (Xrs) by delivering a mild oscillatory pressure of 1-3 cmH_2O waveform at the mouth across different frequencies (i.e., 5Hz, 11Hz, and 19Hz) during tidal breathing. Airway Rrs refers to the frictional forces opposing airflow whereas Xrs measures the elastic and inertial properties of the airways. Simply, Rrs provides metrics on the level of airway obstruction and Xrs evaluates how effectively the airways can be ventilated. The FOT is novel in that both Rrs and Xrs are frequency dependent, wherein lower frequencies (e.g., 5Hz) are more sensitive to changes in the airway periphery whereas higher frequencies (e.g., 19Hz) are more sensitive to changes in the central airways. Forced oscillation of the airways (FOT) measures were taken at the baseline, midpoint, post, and recovery time periods.

Spirometry measures were comprised of forced vital capacity (FVC) and slow vital capacity (SVC) (MedGraphics Cardiorespiratory Diagnostic System, Medical Graphics Corporation) and

conducted according to American Thoracic Society (ATS) recommendations. Forced vital capacity (FVC) is defined the volume delivered during an expiration made as forcefully and completely as possible starting from full inspiration [22]. To perform the FVC, the participant was placed on a mouthpiece with their lips sealed around the mouthpiece and with a nose clip. Participants were instructed to inhale rapidly and completely from functional residual capacity (FRC) and with minimal hesitation was prompted to "blast" the air from their lungs and encouraged to fully exhale. End of test criterion were achieved when the volume-time curve shows no change in volume (<0.025 L) for >1s [22]. The SVC maneuver was performed by starting a maximal inhalation to total lung capacity (TLC) followed by a maximal exhalation [23]. As opposed to the FVC, participants were instructed to inhale and exhale at a comfortable pace (i.e., allowing chest wall recoil to drive the exhalation and not "blasting" out the air nor "holding back" on the exhalation). End of test criterion was defined similarly to the FVC maneuver.

Respiratory pattern data were monitored continuously during both exposures (MedGraphics Cardiorespiratory Diagnostic System, Medical Graphics Corporation) and consisted of tidal volume (Vt), respiratory rate (RR), and minute ventilation (VE). Tidal volume is defined as the amount of air inspired and expired during a single breath and reported in mL; respiratory rate is defined as the number of breaths taken in a minute and reported in breaths/min; and minute ventilation is defined as the amount of air inspired and expired in 1 minute and reported as L/min.

3.3.2 Gas Exchange

Pulmonary blood flow (Vc) and alveolar – capillary conductance (Dm) were calculated from the lung diffusing capacity for carbon monoxide (DLCO) and the lung diffusing capacity for nitric oxide (DLNO). Lung diffusion capacities were assessed using a rebreathe technique by taking

advantage of the diffusion-limited nature of CO and NO gas [24, 25]. Briefly, DLCO and DLNO were determined via the rate of disappearance of CO and NO, respectively. Following a normal expiration, subjects were switched into a rebreathe bag containing the test gas mixture (9% He, 0.3% $C_{18}O$, 35% O_2, and balance N_2) and instructed to nearly empty the bag with each breath for 10 consecutive breaths. The ratio of DLNO to DLCO (termed α ratio) has previously been determined as 2.26 [25]. Additionally, Dm was normalized to Vc to provide a blood volume-independent measure of membrane conductance [26].

Alveolar – capillary conductance (Dm)

$$Dm = \frac{\alpha\ ratio}{DLNO} \quad \textbf{1}$$

Pulmonary blood flow (Vc)

$$Vc = \frac{1}{\theta CO \cdot \left(\left(\frac{1}{DLCO}\right) - \left(\frac{1}{Dm}\right)\right)} \quad \textbf{2}$$

Lung Clearance Index was determined via a N_2 washin washout method. This was measured during the rebreathe technique when small amounts of N_2 were introduced into the breathing circuit. During the rebreathe technique, all participants achieved N_2 washin equilibrium ($F_{i,eq}$) and the washout time was determined as the amount of expired volume necessary to reach $1/40^{th}$ of $F_{i,eq}$. Volumes were converted to STPD for calculations.

Total systemic volume ($V_{s,tot}$):

$$V_{s,tot} = \frac{F_i^0}{F_{i,eq}} \cdot V_{rb} \quad \textbf{3}$$

Functional residual capacity (FRC):

$$FRC = V_{s,tot} - (V_{rb} + V_{ds,rb} + V_{ds}) \quad \textbf{4}$$

Cumulative net volume expired during the multi -breath washout:

$$V_{CE} = \sum_{n=1}^{N} (V_T(n) - V_{DS})) + \frac{Cet(N)/Cet(Start) - 1/40}{Cet(N)/Cet(Start) - Cet(N+1)/Cet(Start)} (V_T(N+1) - V_{DS}) \quad \textbf{5}$$

Lung Clearance Index (LCI):

$$LCI = \frac{V_{CE}}{FRC} \quad \textbf{6}$$

The volume of the rebreathe bag (V_{rb}) was determined during calibration, dead space volume of the rebreathe bag ($V_{ds,rb}$) was assumed to be 0mL, and the dead space volume (V_{ds}) was provided by the manufacturer of the pneumotach and switching valve at 150mL.

Arterial blood draws were taken during resting breathing on the mouthpiece to obtain respiratory exchange equivalent and patterns, $P_{ET}O_2$, and $P_{ET}CO_2$ for ABG calculations (MedGraphics Cardiorespiratory Diagnostic System, Medical Graphics Corporation). Arterial blood was drawn into an evacuated heparinized syringe. Any air bubble in the sample was expelled as soon as possible after withdrawing the sample and before mixing with heparin or before any cooling of the sample was done. Sufficient mixing was achieved via inverting the syringe 10 times and rolling it between the palms to prevent coagulation and stacking of red blood cells. Blood draws were performed at baseline, midpoint, post, and recovery testing periods and taken in duplicates. All blood draws were analyzed within 1 minute of the draw and analyzed in duplicates. The following calculations were used to determine ABGs:

Ratio of physiologic dead space over tidal volume (VD/VT):

$$\frac{VD}{VT} = \frac{PaCO_2 - PECO_2}{PaCO_2} \quad (7)$$

Alveolar-arterial difference (A-a Difference):

$$A - a\ Gradient = PACO_2 - PaO_2 \quad (8)$$

Alveolar partial pressure of oxygen (PAO_2):

$$PAO_2 = (FiO_2)\ x\ (Pb - pH_2O) - (PCO_2\ x \frac{1 - FiO_2\ x\ (1 - RQ)}{RQ}) \quad (9)$$

Additional measures analyzed were pH, hemoglobin concentration (Hb) (g/dL), hematocrit (Hct), respiratory equivalent (RQ), arterial partial pressure of oxygen (PaO_2), and arterial partial pressure of carbon dioxide ($PaCO_2$).

A lung ultrasound was taken (Philips Lumify Ultrasound, Philips Healthcare Systems) utilizing 28 sonographic windows as defined by Summerfield at al. [27]. A comet tail was defined as hyperechoic reflections at regions of high acoustic mismatch, signaling increase fluid content. Comet tail clusters were counted as 5 comet tails for consistent quantification.

3.3.3 Hemodynamics

Cardiac output (Q) was measured during the rebreathe technique via assessment of the disappearance rate of C_2H_2. C_2H_2 is an inert, soluble gas that enters the blood stream via pulmonary diffusion but does not bind to hemoglobin. Therefore, the disappearance rate of C_2H_2 is proportional to pulmonary blood flow and in participants without lung disease, the pulmonary

blood flow is equal to systemic blood flow [28]. Stroke volume (SV) was then determine via continuously monitored HR.

The partial pressures of O_2 and CO_2 ($P_{ET}O_2$ and $P_{ET}CO_2$, respectively) were measured via a mass spectrometer (Marquette 110 Medical Gas Analyzer Mass Spectrometer, Perkins Elmer; Sievers 280i Nitric Oxide Analyzer, GE Analytical Instruments) from a sample line placed in the expiratory limb of the experimental breathing circuit. Pulse oxygenation was measured via the forehead (Radical 7, Massimo, CA, USA). Heart rate and rhythm was recorded using a single-channel bio-amplifier module (FE132, ADInstruments, NSW, AUS). Arterial BP was continuously monitored via the arterial catheter.

Cerebral oxygenation (RSO_2) was obtained via near-infrared spectroscopy (NIRS) (SenSmart Model X-100, Nonin) was sampled at the forehead and continuously monitored. Transcranial doppler (Multigon Industries, Yonkers, NY) was used to monitor CBF via blood flow velocities in the major basal intracranial arteries. This was monitored continuously while undergoing testing.

3.3.4 Symptoms

A proprietary scale was developed to evaluate subjective rating of respiratory and cognitive symptoms during the exposure (**Table 1**). Participants were asked to rate their symptoms on a scale from 1-5. As the participants were instructed to remain on the mouthpiece for the duration of the exposure, they were asked to provide their rating by holding up the number of fingers corresponding to their symptomology. Following the exposure, participants were given the opportunity to elaborate on an

Table 1. Rating scale to assess respiratory and cognitive symptoms

Respiratory Symptoms	
Chest Tightness	
Desire to Cough	0 - None
Other	1 - Little
	2 - Little/Moderate
Cognitive Symptoms	3 - Moderate
Lightheaded	4 - Moderate/Severe
Confusion	5 - Severe
Vision Changes	
Other	

3.4 Statistical Approach

The measured and computed variables obtained during the baseline, midpoint, post, and recovery time periods were averaged to provide a single value per time period. Repeated measures analyses of variance (ANOVAs) were used to determine the effect of exposure to oscillating O_2/N_2 concentrations in the background of mild altitude on respiratory mechanics, gas exchange, and hemodynamics. Data obtained during the chamber visit were analyzed using separate repeated measures ANOVAs.

Generalized additive mixed effects models (GAMMs) were used to determine the impact of the duration of the exposure on respiratory mechanics, gas exchange, hemodynamics, and cognitive performance. In brief, the GAMM model is similar to multiple linear regressions, insofar as it attempts to model the independent (main) effects of inputted covariates on the outcome variable

(e.g., $P_{ET}CO_2$). However, an important assumption of multiple linear regression is that all observations are independent, uncorrelated with each other, and demonstrate linearity – an assumption that is flatly violated with repeated measures data, such as in our study. The GAMM model, on the other hand, handles repeated observations by robustly modelling the correlation between observations clustered within each subject. Additionally, the GAMM allows for the fitting of non-linear smoothing splines to individual, repeated measures. Thus, the GAMM model allows for non-linear modeling and provides group-level parameter estimates of covariate main-effects after accounting for the within-subject correlation between observations.

The GAMM models used in this study were selected through the interrogation of multiple competing models. Additionally, competing distributional families were compared using the Akaike information criterion (AIC) to determine which family was most appropriate. Through these comparisons, we determined a log linked Gamma distribution most closely fit the data for the reaction time models and a beta regression family most closely fit the data for the error rate models. Included in these models were main effects and random effects for patient ID, heart rate, systolic BP, thoracic impedance, CBF and oxygenation, and $PETCO_2$. The selection of group-level main effects and interaction terms was determined using a backward selection method based on the Akaike's Information Criteria (AIC) score [29]. The final GAMM model was fit using the restricted maximal likelihood (REML) method, cubic regression penalties for nonlinear smooths, the hyperparameter γ was calculated using BIC-like parameters (i.e., log(n)/2) to reduce overfitting, and a false discover p-value adjustment to reduce false positives [30, 31]. An extra penalty was added to each individual term so it could be penalized to zero, thereby allowing terms to be automatically "selected out" from the GAMM when appropriate. Statistical significance was considered if $P < 0.05$.

4.0 RESULTS AND DISCUSSION

4.1 Subject Characteristics, Symptoms, and Comet Tails

While there was variability within oscillation rates, there were no significant differences between the 3 groups based on oscillation rates (e.g., 30s oscillations vs. 60s oscillations) for subject age, height, weight, or BMI (**Table 2**). Furthermore, there were no significant changes in symptoms ratings for either respiratory or cognitive symptoms across the exposure (**Table 3**). While it may appear there are interesting, inter-oscillation trends evident in the symptoms recorded, it must be noted any variation from a "0" rating (i.e., no symptoms at all) can be attributed primarily to a single participant in the 30s and 60s oscillation rates. On the other hand, there were more participants who rated themselves as having symptoms in the 120s oscillation group compared with the 30s and 60s oscillations, albeit still minimal symptoms. As such, the most robust finding in our symptoms data is that while no participants gave a symptom rating above a 3 (i.e., Moderate), there were more participants within the 120s oscillation group that rated as having *any* symptoms in response to the exposure. It must also be noted that the "symptoms" rated in the "Other category consisted primarily of a tension sensation or headache attributed to the TCD cap. Additionally, we observed no statistical differences in the number of comet tails observed pre and post exposure (5.9 vs 7.0, 6.4 vs 7.6, and 7.1 vs 9.6 for 30s, 60s, and 120s oscillations respectively). However, there does appear to be a trend towards increased comet tails, the magnitude of which is larger for longer oscillation rates.

Table 2. Subject characteristics.

	N (females)	Mean	SD
All Subjects			
Age (yr)	30 (7)	28.93	5.30
Height (cm)	30 (7)	175.54	10.48
Weight (kg)	30 (7)	76.56	11.21
BMI	30 (7)	24.79	2.34
30s Oscillation Subjects			
Age	10 (2)	30.00	6.02
Height	10 (2)	172.76	7.20
Weight	10 (2)	75.61	8.38
BMI	10 (2)	25.34	2.43
60s Oscillation Subjects			
Age	9 (3)	27.30	5.06
Height	9 (3)	176.50	14.14
Weight	9 (3)	75.83	15.10
BMI	9 (3)	24.17	2.01
120s Oscillation Subjects			
Age	10 (2)	29.50	4.90
Height	10 (2)	177.35	9.43
Weight	10 (2)	78.24	10.10
BMI	10 (2)	24.87	2.64

SD: standard deviation

Table 3. Symptoms ratings

	Testing Time (min)											
	0	**8**	**16**	**24**	**32**	**40**	**45**	**53**	**61**	**69**	**77**	**85**
30s Oscillations												
Respiratory Symptoms												
Chest Tightness	0	0	0	0	0	0	0	0	0	0	0	0
Desire to Cough	0	0	0	0	0	0	0	0	0	0.1	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0
Cognitive Symptoms												
Lightheadedness	0	0.1	0.1	0.1	0.1	0.1	0	0	0	0.1	0.1	0.1
Confusion	0	0	0	0	0	0	0	0	0	0	0	0
Vision	0	0.1	0	0	0	0	0	0	0	0	0.1	0
Other	0	0	0	0	0	0	0	0	0	0	0	0
60s Oscillations												
Respiratory Symptoms												
Chest Tightness	0	0	0	0	0	0	0	0	0	0	0	0
Desire to Cough	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0	0	0	0	0	0	0	0	0	0	0
Cognitive Symptoms												
Lightheadedness	0	0.1	0	0.2	0.3	0.2	0	0	0.1	0	0	0
Confusion	0	0.1	0	0	0	0.1	0	0	0	0	0	0
Vision	0	0	0	0	0	0	0	0	0	0	0	0
Other	0	0.1	0.2	0.1	0.1	0.1	0	0	0.1	0.2	0	0
120s Oscillations												
Respiratory Symptoms												
Chest Tightness	0	0	0	0	0	0	0	0	0	0	0	0
Desire to Cough	0	0	0.2	0.1	0.2	0.2	0	0	0	0.1	0.1	0
Other	0	0	0	0	0	0	0	0	0	0	0	0
Cognitive Symptoms												
Lightheadedness	0.1	0.1	0.2	0.1	0.2	0.4	0	0	0	0.1	0.3	0.3
Confusion	0	0.1	0.1	0.2	0.2	0.1	0	0.1	0	0.2	0.2	0.2
Vision	0	0	0	0	0.1	0.1	0	0	0	0.1	0.1	0
Other	0	0	0.2	0.2	0.3	0.5	0	0	0	0.1	0.2	0.4

4.2 Respiratory Mechanics and Airway Inflammation

4.2.1 Fractional Exhaled Nitric Oxide

We did observe a systematic decrease in F_ENO measures across the testing periods with an increase towards baseline at the recovery testing period for all oscillation rates (i.e., 30s, 60s, and 120s). However, none of these changes were statistically significant (**Supplementary Table 1**). These data suggest being exposed to high inspired O_2 at varying oscillation rates do not induce airway inflammation as measured by F_ENO. We propose two mechanisms whereby we observed these non-significant decreases in F_ENO. First, previous literature has clearly demonstrated a majority of NO production occurs in the paranasal cavity [32, 33]. Indeed, oscillatory airflow through nasal cavity has been shown to significantly increases NO production, suggesting expiratory flow rates through the nasal cavity is a strong trigger of NO production [34]. However, there is a well-accepted 2-compartment model wherein NO is produced via inflammatory epithelial cells in both the upper and lower respiratory tracts. Within this model, nasal congestion in the presence of airway inflammation results in increased NO production in inflammatory cells within the lower respiratory tract [35]. Thus, it is reasonable that the decrease in F_ENO we observed in response to extended mouth breathing imposed upon the participant suggests the decreased nasal flow rates suppressed NO production in paranasal cavity in the absence of the upregulation of NO production in response to inflammation. Second, an underappreciated factor, not accounted for in the 2-compartment model, that may influence exhaled NO is axial diffusion [36]. Previous literature has demonstrated decreased BP (as demonstrated in the current study; see below) may increase axial back diffusion of NO in the alveolar compartment [36]. This increased axial back diffusion coupled with the strong affinity of NO to hemoglobin [37] may explain the decreases in F_ENO.

4.2.2 Forced Oscillation Technique

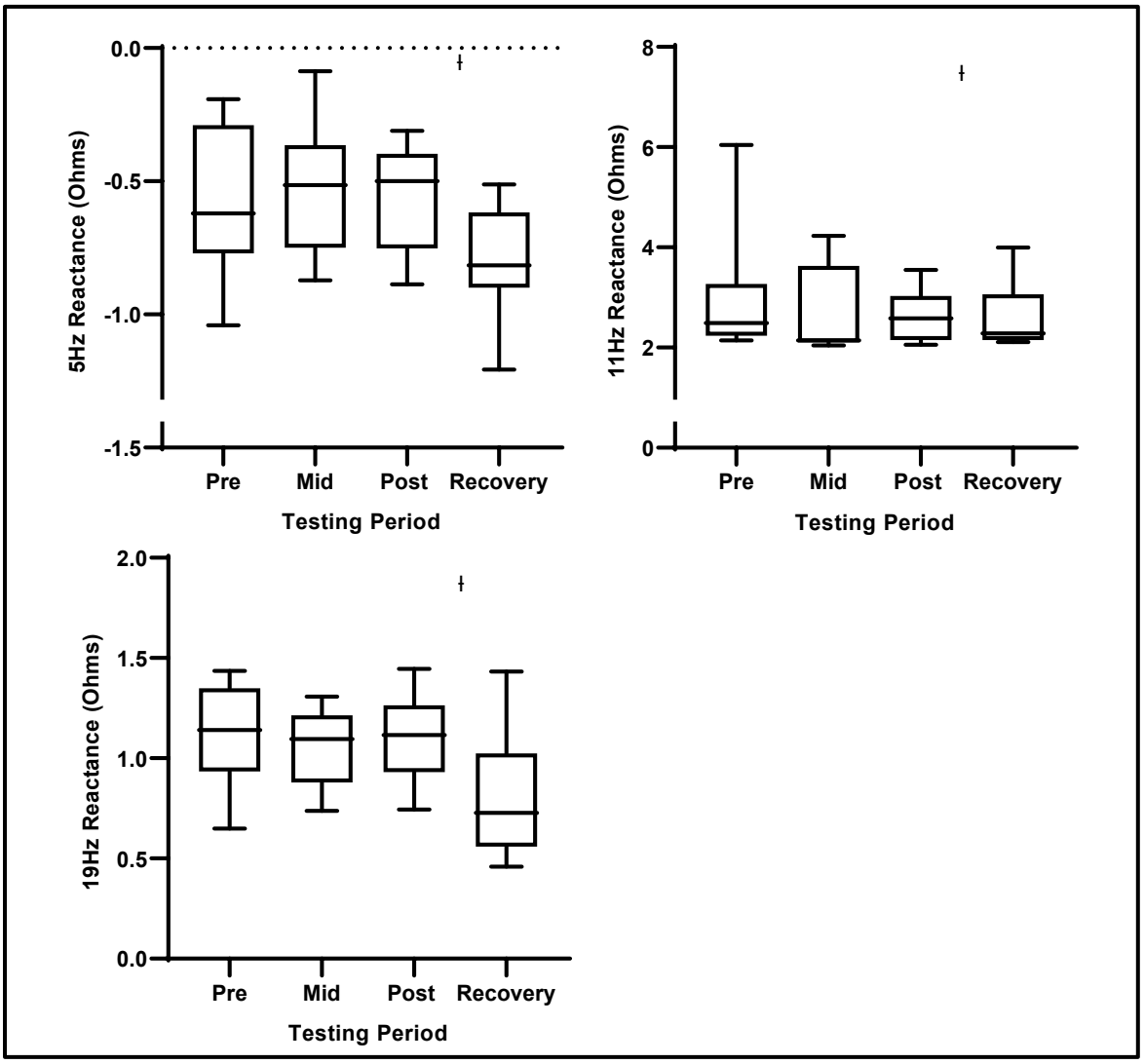
During the 30s oscillations, there was no evidence of changes in airway Rrs at any FOT frequency (5Hz, 11Hz, and 19Hz). On the other hand, there were systematic decreases in airway Xrs across all frequencies (**Table 4; Figure 2)**. Specifically, at every frequency, Xrs at the recovery testing period was significantly lower when compared with Xrs at the baseline testing period. This decrease (i.e., more negative) Xrs coupled with normal Rrs suggests alterations to the peripheral airways. In fact, a more negative Xrs has been shown in patients suffering from interstitial lung disease [38]. This relationship between Xrs and Rrs may be indicative of airway obstruction resulting from alveolar atelectasis and dis-homogeneity of ventilation. Simply, as peripheral airway units collapse and "drop off," one would expect to see a decrease in the system's capacitance, as it has essentially lost a portion of surface area that contributes elasticity to the pulmonary structure. Indeed, these results are consistent with the presence of peripheral airway inflammation and ventilation dis-homogeneity [39]. On the other hand, during both the 60s and 120s oscillations, we did not observe any changes in airway resistance and reactance (**Supplementary Table 2; Supplementary Table 3**). These data suggest a decrease in peripheral airway compliance in response to a 30s oscillatory rate not observed in the 60s and 120s oscillatory rates. This may be a consequence of the more rapid alveolar recruitment-derecruitment cycle associated with the 30s oscillations; and why a similar change in Xrs was not seen in the longer oscillatory rates. It must be noted the magnitude of change in reactance was small and may not constitute physiologic relevancy.

Table 4. The influence of 30s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on airway resistance and reactance at 5, 11, and 19Hz.

Testing Period	Mean	SD	*p-value*
		5Hz Resistance	
Pre	3.00	1.28	
Mid	2.65	0.83	0.41
Post	2.67	0.59	0.49
Recovery	2.72	0.77	0.67
		5Hz Reactance	
Pre	–0.57	0.28	
Mid	–0.53	0.25	0.73
Post	–0.56	0.20	0.99
Recovery	–0.79	0.21	**0.02**
		11Hz Resistance	
Pre	2.97	1.26	
Mid	2.71	0.89	0.70
Post	2.62	0.52	0.51
Recovery	2.61	0.66	0.50
		11Hz Reactance	
Pre	0.22	0.16	
Mid	0.25	0.16	0.98
Post	0.24	0.07	0.99
Recovery	0.03	0.19	**0.02**
		19Hz Resistance	
Pre	2.96	1.18	
Mid	2.73	0.87	0.78
Post	2.62	0.49	0.48
Recovery	2.59	0.65	0.41
		19Hz Reactance	
Pre	1.12	0.25	
Mid	1.06	0.20	0.79
Post	1.10	0.22	0.95
Recovery	0.81	0.31	**<0.01**

SD: standard deviation. Resistance and reactance are reported in Ohms. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period ($P < 0.05$).

Figure 2. Airway reactance during the 30s oscillation exposure.



Values represent means ± SD. Ł Significant difference from the Pre, Mid, and Post-testing period, $P < 0.05$.

4.2.3 Pulmonary Function Tests

During the 30s oscillation exposure, there was no effect of the exposure on FVC, forced expiratory volume in 1 second (FEV1), maximal forced expiratory flow (FEF_{max}), or SVC. There was however a significant increase in the inspiratory capacity from the baseline testing to post-testing period ($P < 0.01$), an increase that was not observed in the recovery testing period (**Table 5; Figure 3**). Conversely, there was a significant decrease in the expiratory reserve volume (ERV) at the post-testing period ($P<0.001$) and recover testing period ($P<0.05$) when compared to baseline testing (**Table 5; Figure 3**). These data suggest that the 30s oscillatory rate may result in participants breathing at a lower operational lung volume, as evident by the increased IC in conjunction with the decreased ERV.

When participants were exposed to the 60s oscillations, we observed a systematic decrease in FVC and FEV1 with an improvement towards baseline at the recovery testing period (**Table 6; Figure 4**). Specifically, FVC and FEV1 measures at the mid, post, and recovery period were all significantly lower than at the baseline testing period. We observed a similar trend in SVC and ERV measures in the 60s oscillation cohort as well. While SVC measures at the mid and post-testing period were non-significantly lower than the baseline testing period, SVC measures at the recover testing period *was* significantly lower than baseline SVC. Additionally, there was a systematic decrease in ERV with post-testing ERV measures being significantly lower than baseline. The diminished volumes observed in our 60s oscillation cohort may be indicative of alveolar atelectasis. In fact, alveolar atelectasis is significantly correlated to decreased lung volumes, especially as measured via FVC and FEV1 [40, 41]. It is important to note that these spirometric measures are highly volitional and, thus, may be influenced by participant fatigue.

However, the researchers adhered to ATS standards for conducting pulmonary function testing and provided ample encouragement to the subjects. As such, we are confident the spirometric measures obtained were accurate.

Similarly, during the 120s oscillation exposures, our cohort demonstrated systematically lower FVC, FEV1, FEF_{max}, and SVC with an improvement towards baseline during the 120s oscillation exposure (**Table 7; Figure 5**). Specifically, FVC measures and the mid and post-testing period were significantly lower compared with baseline testing. We also observed a significantly lower FEV1 and SVC at the mid-testing period when compared with baseline testing. Additionally, our cohort produced significantly lower maximal expiratory flows (i.e., FEF_{max}) at the recovery testing period compared to baseline testing. Similar to the 60s oscillations, the decreases in lung volumes (i.e., FVC and FEV1) may be the result of alveolar atelectasis. Furthermore, the reduction in flow rates may also point to small airway impairment. In fact, reduced expiratory flow rates have been demonstrated to be highly correlated to small airway disease [42]. Thus, these data may indicate exposure to the 120s oscillations engendered small airway and alveolar impairment in this cohort.

Table 5. The influence of 30s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on pulmonary function.

Testing Period	Mean	SD	*p-value*
Forced Vital Capacity			
Pre	97.37	8.32	
Mid	95.67	8.05	0.61
Post	94.28	7.27	0.09
Recovery	95.94	7.25	0.76
Forced Expiratory Volume in 1 Second			
Pre	92.93	6.65	
Mid	90.50	5.55	0.41
Post	89.61	6.57	0.11
Recovery	91.39	5.54	0.86
Maximal Forced Expiratory Flow			
Pre	89.83	14.33	
Mid	92.11	14.99	0.91
Post	95.72	14.74	0.14
Recovery	96.50	14.99	0.08
Slow Vital Capacity			
Pre	99.53	9.24	
Mid	97.28	9.37	0.25
Post	96.78	9.97	0.11
Recovery	96.44	10.08	0.06
Inspiratory Capacity			
Pre	106.83	13.88	
Mid	108.39	14.37	0.44
Post	113.11	16.46	**<0.01**
Recovery	107.56	14.77	0.66
Expiratory Reserve Volume			
Pre	86.57	21.12	
Mid	77.78	15.57	0.07
Post	68.39	18.08	**<0.001**
Recovery	76.72	18.59	**0.04**

SD: standard deviation. Mean values are presented as a percent predicted. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 6. The influence of 60s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on pulmonary function.

Testing Period	Mean	SD	*p-value*
Forced Vital Capacity			
Pre	104.23	11.38	
Mid	100.10	10.62	**<0.01**
Post	99.85	10.87	**<0.01**
Recovery	100.10	10.33	**<0.01**
Forced Expiratory Volume in 1 Second			
Pre	98.77	12.29	
Mid	95.35	11.60	**<0.01**
Post	95.15	10.87	**<0.01**
Recovery	95.95	10.88	**<0.05**
Maximal Forced Expiratory Flow			
Pre	92.03	16.13	
Mid	88.40	15.42	0.12
Post	93.10	13.93	0.91
Recovery	90.75	16.48	0.85
Slow Vital Capacity			
Pre	109.07	10.72	
Mid	107.30	11.42	0.65
Post	107.35	12.11	0.67
Recovery	104.55	11.35	**<0.05**
Inspiratory Capacity			
Pre	126.13	24.09	
Mid	131.05	24.67	0.60
Post	136.15	26.64	0.08
Recovery	127.60	27.56	0.98
Expiratory Reserve Volume			
Pre	82.10	23.25	
Mid	68.15	32.78	0.06
Post	60.65	24.39	**<0.01**
Recovery	68.30	28.53	0.07

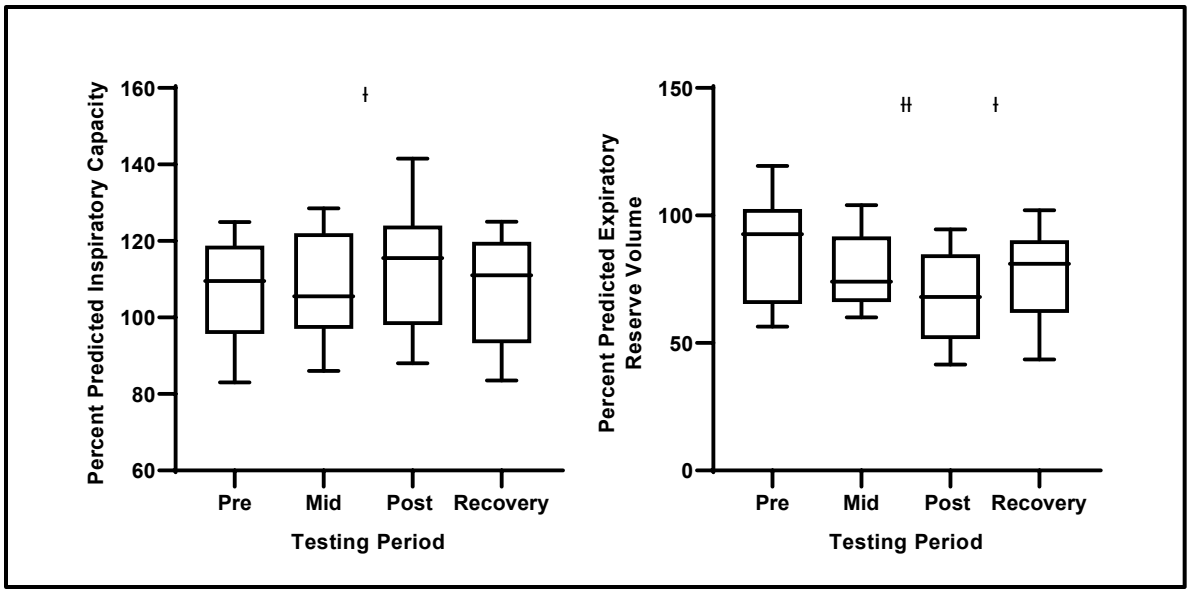
SD: standard deviation. Mean values are presented as a percent predicted. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period ($P < 0.05$).

Table 7. The influence of 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on pulmonary function.

Testing Period	Mean	SD	*p-value*
Forced Vital Capacity			
Pre	105.15	5.44	
Mid	102.59	5.28	**<0.01**
Post	102.27	5.58	**<0.01**
Recovery	104.09	5.78	0.44
Forced Expiratory Volume in 1 Second			
Pre	96.49	10.33	
Mid	93.10	8.39	**0.02**
Post	94.33	11.35	0.18
Recovery	96.11	10.66	0.98
Maximal Forced Expiratory Flow			
Pre	88.26	9.19	
Mid	85.89	7.33	0.59
Post	85.94	7.83	0.61
Recovery	81.94	10.93	**0.01**
Slow Vital Capacity			
Pre	108.30	4.83	
Mid	105.25	4.29	**0.03**
Post	105.90	5.75	0.12
Recovery	106.15	4.63	0.19
Inspiratory Capacity			
Pre	122.90	12.74	
Mid	121.40	7.66	0.93
Post	125.10	11.70	0.80
Recovery	123.30	12.27	0.99
Expiratory Reserve Volume			
Pre	82.47	20.92	
Mid	77.00	12.41	0.71
Post	76.90	14.14	0.69
Recovery	79.40	11.57	0.93

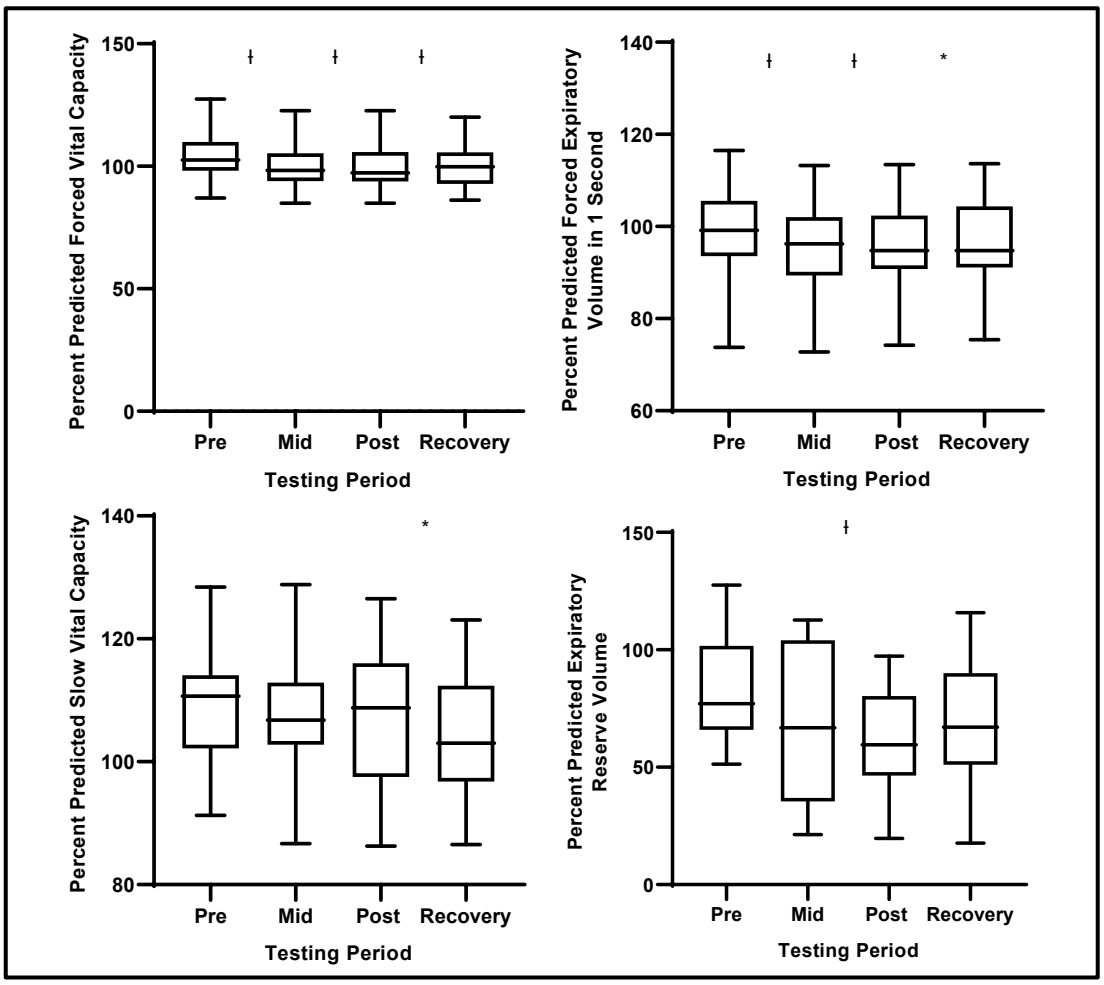
SD: standard deviation. Mean values are presented as a percent predicted. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Figure 3. Inspiratory capacity and expiratory reserve volume during the 30s oscillation exposure.



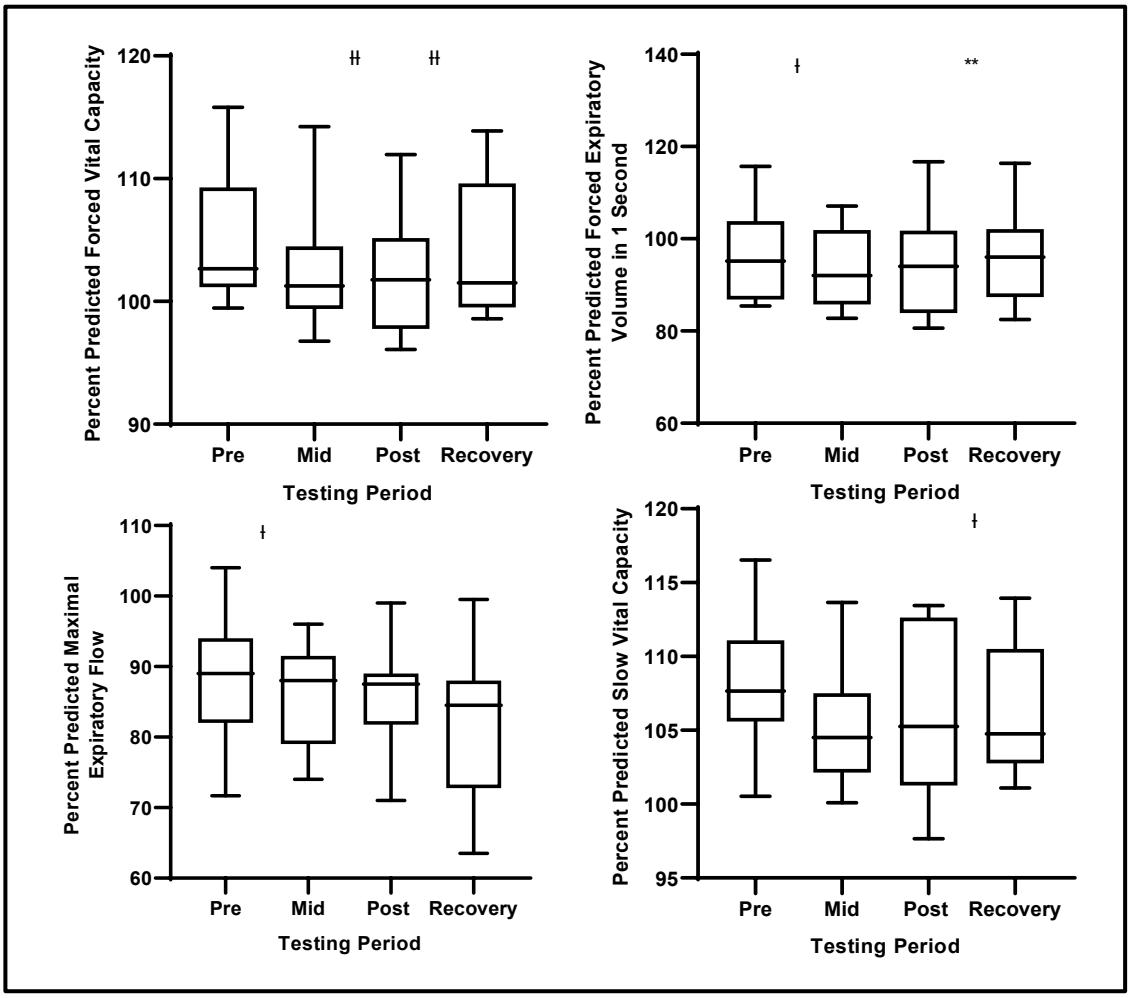
Values represent means ± SD. Ɨ Significant difference from the Pretesting period, $P < 0.05$. Ⱨ Significant difference from the Pretesting period, $P < 0.001$

Figure 4. Forced vital capacity, forced expiratory volume in 1 second, slow vital capacity, and expiratory reserve volume during the 60s oscillation exposure.



Values represent means ± SD. * Significant difference from the Pretesting period, $P < 0.05$. Ł Significant difference from the Pretesting period, $P < 0.01$.

Figure 5. Forced vital capacity, forced expiratory volume in 1 second, maximal forced expiratory flow, and slow vital capacity during the 120s oscillation exposure.

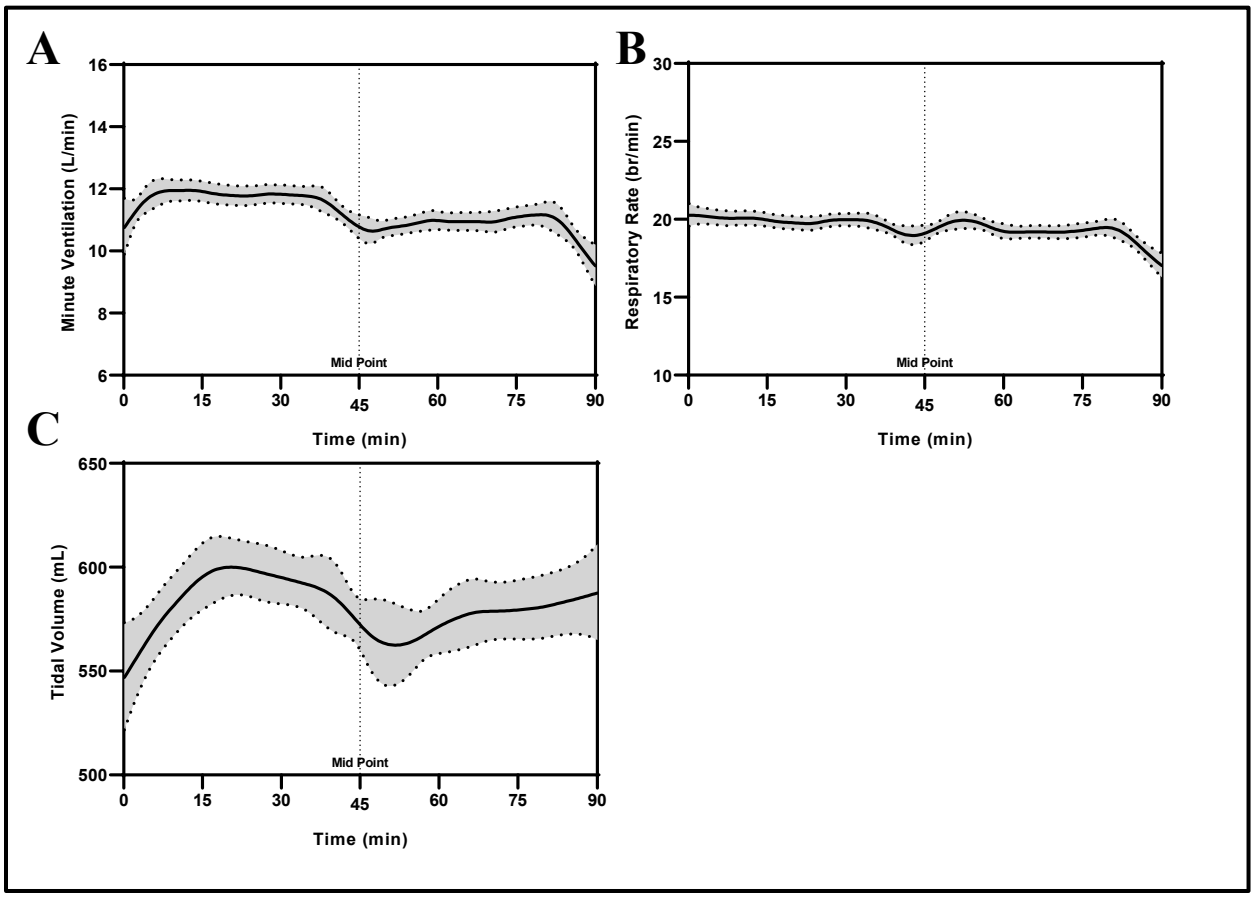


Values represent means ± SD. Ɨ Significant difference from the Pretesting period, $P < 0.05$. ǂ Significant difference from the Pretesting period, $P < 0.01$. ** Significant difference from the Mid-testing period, $P < 0.05$.

4.2.4 Respiratory Patterns

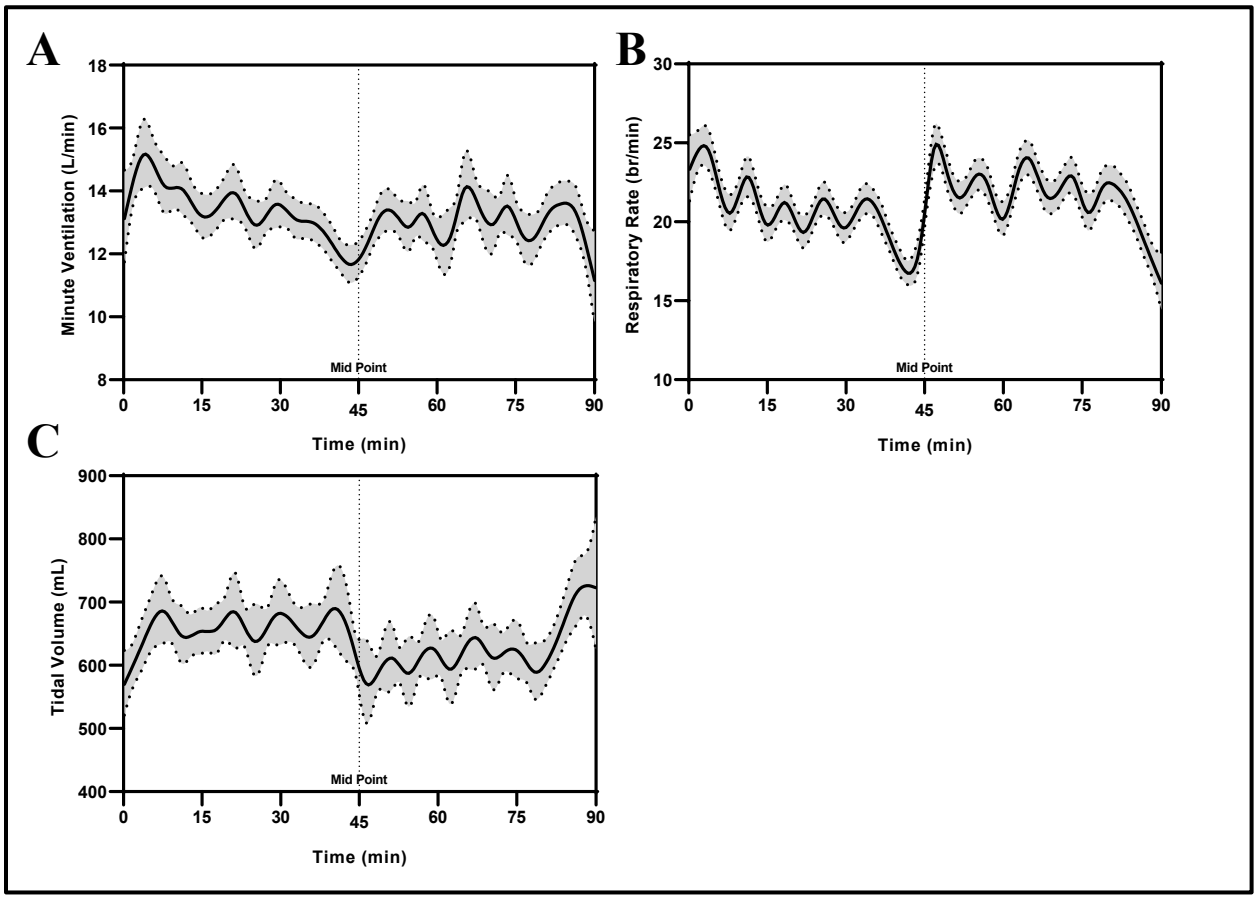
We observed no change in VE, RR, or Vt in response to the 30s oscillation exposure (**Figure 6**). Similarly, there were no changes in VE during the 120s oscillations. However, our data did demonstrate a significant decrease in RR and a compensatory, non-significant increase in Vt (P = 0.06) (**Figure 7**). These data suggest longer exposures to hyperoxia (120s vs 30s oscillations) may depress RR and increase Vt, sufficient for maintenance of VE. We observed a similar trend in respiratory patterns during the 60s oscillations insofar as RR was dampened and Vt was increased (**Figure 8**). However, in contrast to the 120s oscillation exposures, VE did decrease during the 60s oscillations. While historically, exposure to hyperoxia induces marked increases in VE, recent literature suggests VE may not be altered in response to hyperoxia [43, 44]. This relationship between hyperoxia and VE is also present in hypobaria, wherein hyperoxic hypobaria has been shown to produce less exaggerated VE response to exercise compared with hyperoxic normobaria [45]. Furthermore, changes in VE and PetCO2 in response to normoxic hypobaria were minimal relative to normoxic normobaria [46]. Taken together, these data suggest exposure to hyperoxic hypobaria may have little effect on respiratory patterns but that longer exposures to oscillating hyperoxia may exert a more pronounced effect on respiratory patterns.

Figure 6. The nonlinear trends in respiratory patterns during the 30s oscillation exposure.



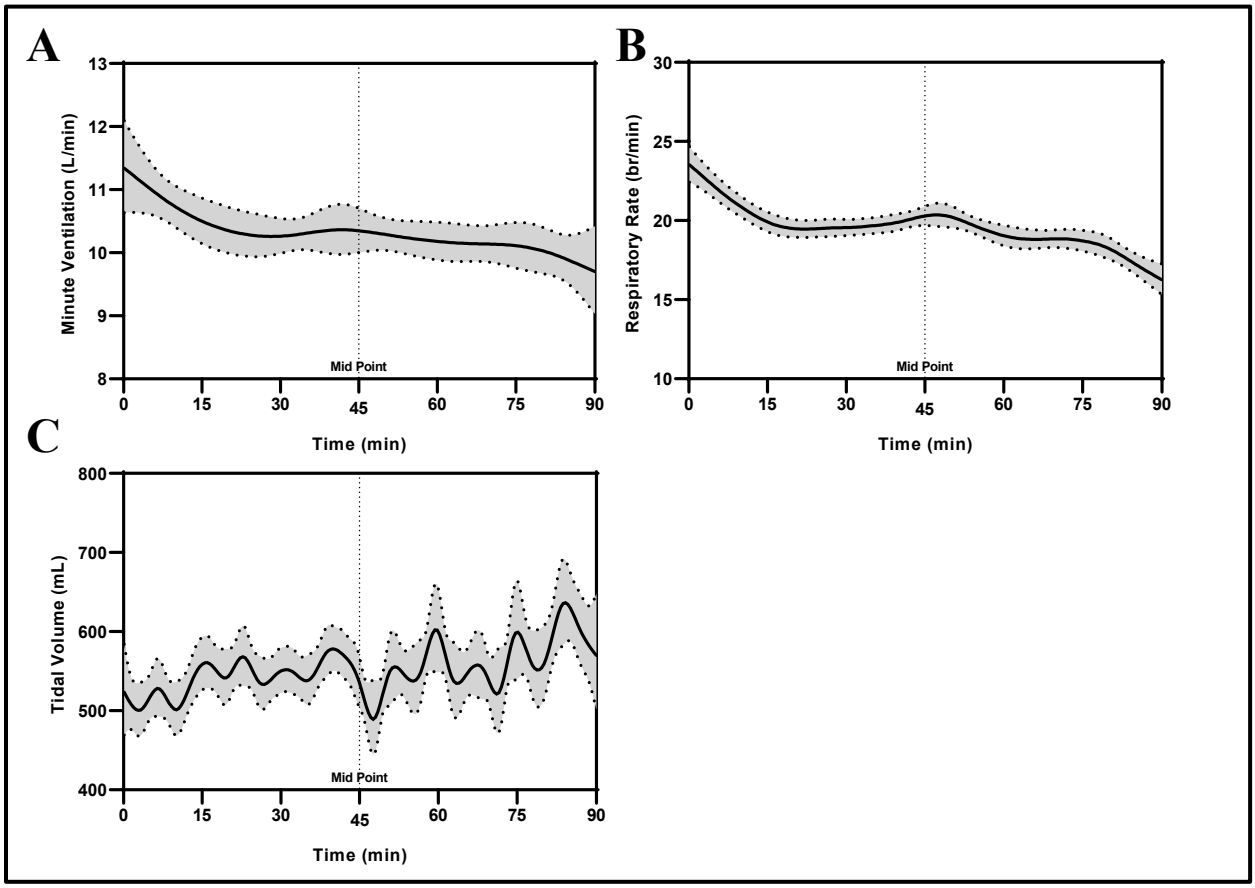
The non-linear trends in respiratory patterns. The shaded regions reference the SD. **Panel A** depicts minute ventilation (L/min); **Panel B** depicts respiratory rate (br/min); and **Panel C** depicts tidal volume (mL).

Figure 7. The nonlinear trends in respiratory patterns during the 60s oscillation exposure.



The non-linear trends in respiratory patterns. The shaded regions reference the SD. **Panel A** depicts minute ventilation (L/min); **Panel B** depicts respiratory rate (br/min); and **Panel C** depicts tidal volume (mL).

Figure 8. The nonlinear trends in respiratory patterns during the 120s oscillation exposure.



The non-linear trends in respiratory patterns. The shaded regions reference the SD. **Panel A** depicts minute ventilation (L/min); **Panel B** depicts respiratory rate (br/min); and **Panel C** depicts tidal volume (mL).

4.3 Gas Exchange

4.3.1 Membrane Diffusion Capacity and Pulmonary-Capillary Blood Volume

When exposed to the 30s oscillations, there were no differences in Dm at any testing period in our cohort. Further there was no difference in the Dm to Vc ratio. We observed a significantly reduced Vc at the post-testing period when compared to baseline testing (**Table 8; Figure 9)**. A concomitant reduction in Q at the post-testing period was also observed. These data suggest exposure to oscillating inspired O_2/N_2 ratios may cause mild pulmonary vascular derecruitment, reduced distention and/or mild pulmonary-capillary vasoconstriction. Additionally, we observed a systematic decline in Q, further supporting pulmonary vascular derecruitment. It is important to note, we cannot be sure if this reduction in Vc is a result of systemically reduced Q or pulmonary vascular vasoconstriction in response to high inspired O_2 concentrations.

During the 60s oscillations, we observed no changes in any of our gas diffusion or Q parameters (**Supplementary Table 4**). On the other hand, exposure to the 120s oscillations resulted in a significant decrease in Vc at the recovery testing period when compared with baseline testing (**Table 9; Figure 10**). While there was no difference in Dm at any testing period, there was an increase in the ratio of Dm and Vc at both the post and recovery period compared with baseline testing (**Table 9; Figure 10**). Our cohort demonstrated possible pulmonary capillary vasoconstriction resulting in an increased Dm/Vc. These data suggest exposure to 120s oscillations of high inspired O_2 may result in pulmonary capillary vasoconstriction and a slight thinning of the alveolar-capillary membrane.

Table 8. The influence of 30s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on lung diffusion measures.

Testing Period	Mean	SD	*p-value*
Membrane Diffusion Capacity (mL/min/mmHg)			
Pre	43.09	8.27	
Mid	42.31	9.13	0.81
Post	43.29	8.19	0.99
Recovery	43.57	9.93	0.99
Pulmonary-Capillary Blood Volume (mL)			
Pre	107.58	25.34	
Mid	100.49	14.98	0.34
Post	94.30	17.86	**0.03**
Recovery	97.47	18.82	0.11
Dm/Vc (1/min/mmHg)			
Pre	0.41	0.08	
Mid	0.45	0.08	0.99
Post	0.49	0.10	0.29
Recovery	0.48	0.12	0.43
Cardiac Output (L/min)			
Pre	5.48	1.02	
Mid	5.16	0.98	0.34
Post	4.88	0.83	**0.03**
Recovery	5.01	0.97	0.10

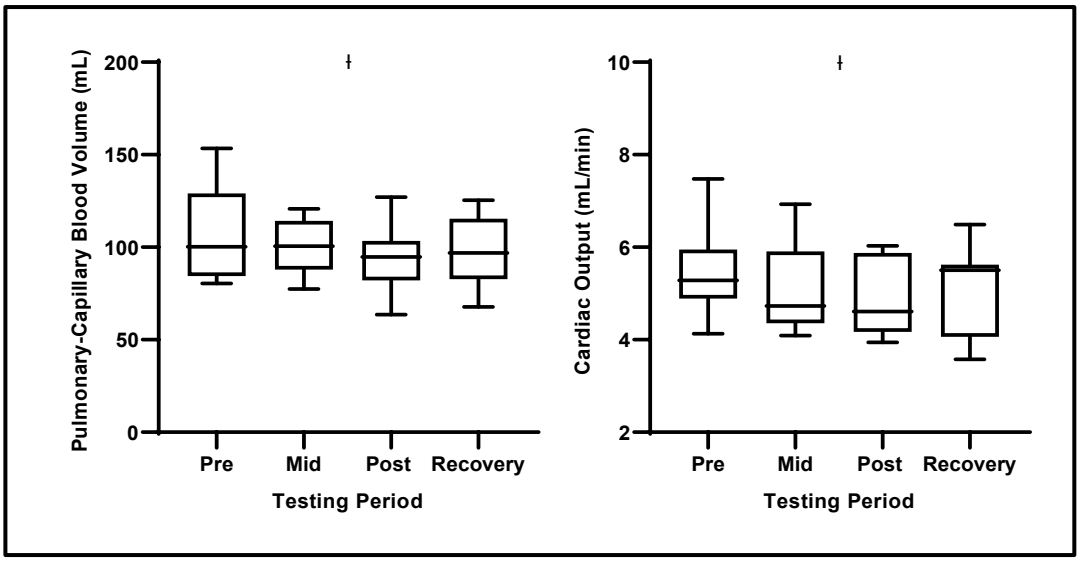
SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period ($P < 0.05$).

Table 9. The influence of 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on lung diffusion measures.

Testing Period	Mean	SD	*p-value*
Membrane Diffusion Capacity (mL/min/mmHg)			
Pre	38.95	8.09	
Mid	41.43	7.54	0.39
Post	42.88	8.93	0.08
Recovery	42.25	9.88	0.17
Pulmonary-Capillary Blood Volume (mL)			
Pre	119.70	22.18	
Mid	112.16	27.14	0.51
Post	104.97	20.76	0.06
Recovery	100.89	16.00	**<0.01**
Dm/Vc (1/min/mmHg)			
Pre	0.34	0.07	
Mid	0.39	0.09	0.14
Post	0.43	0.09	**<0.01**
Recovery	0.44	0.06	**<0.01**
Cardiac Output (L/min)			
Pre	5.52	1.04	
Mid	5.31	0.77	0.80
Post	5.31	0.78	0.79
Recovery	5.37	0.93	0.90

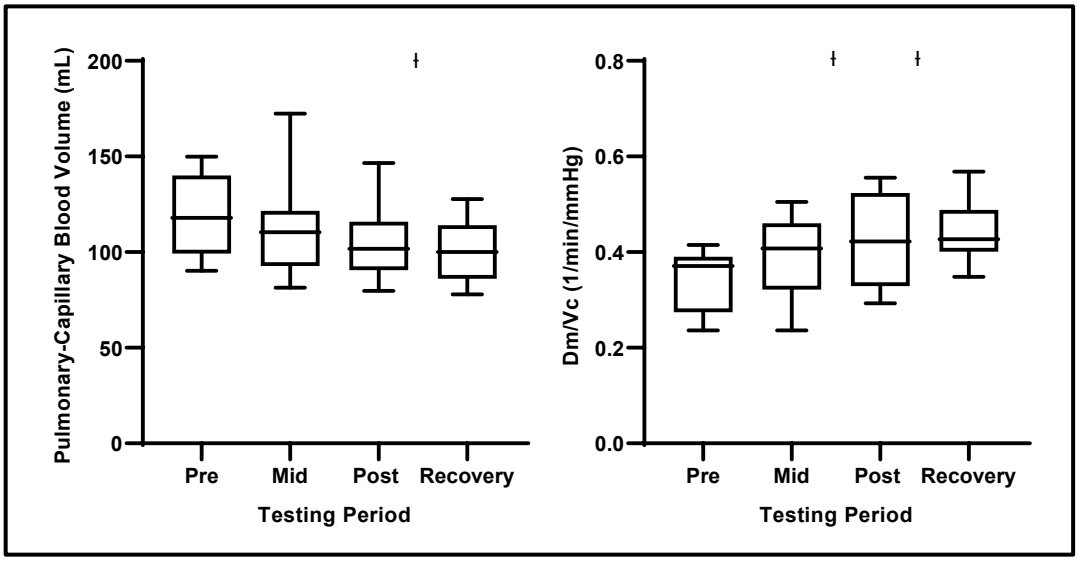
SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Figure 9. Pulmonary-capillary blood volume and cardiac output during the 30s oscillation exposure.



Values represent means ± SD. Ɨ Significant difference from the Pretesting period, $P < 0.05$.

Figure 10. Pulmonary-capillary blood volume and the ratio between membrane diffusion capacity and pulmonary-capillary blood volume during the 120s oscillation exposure.

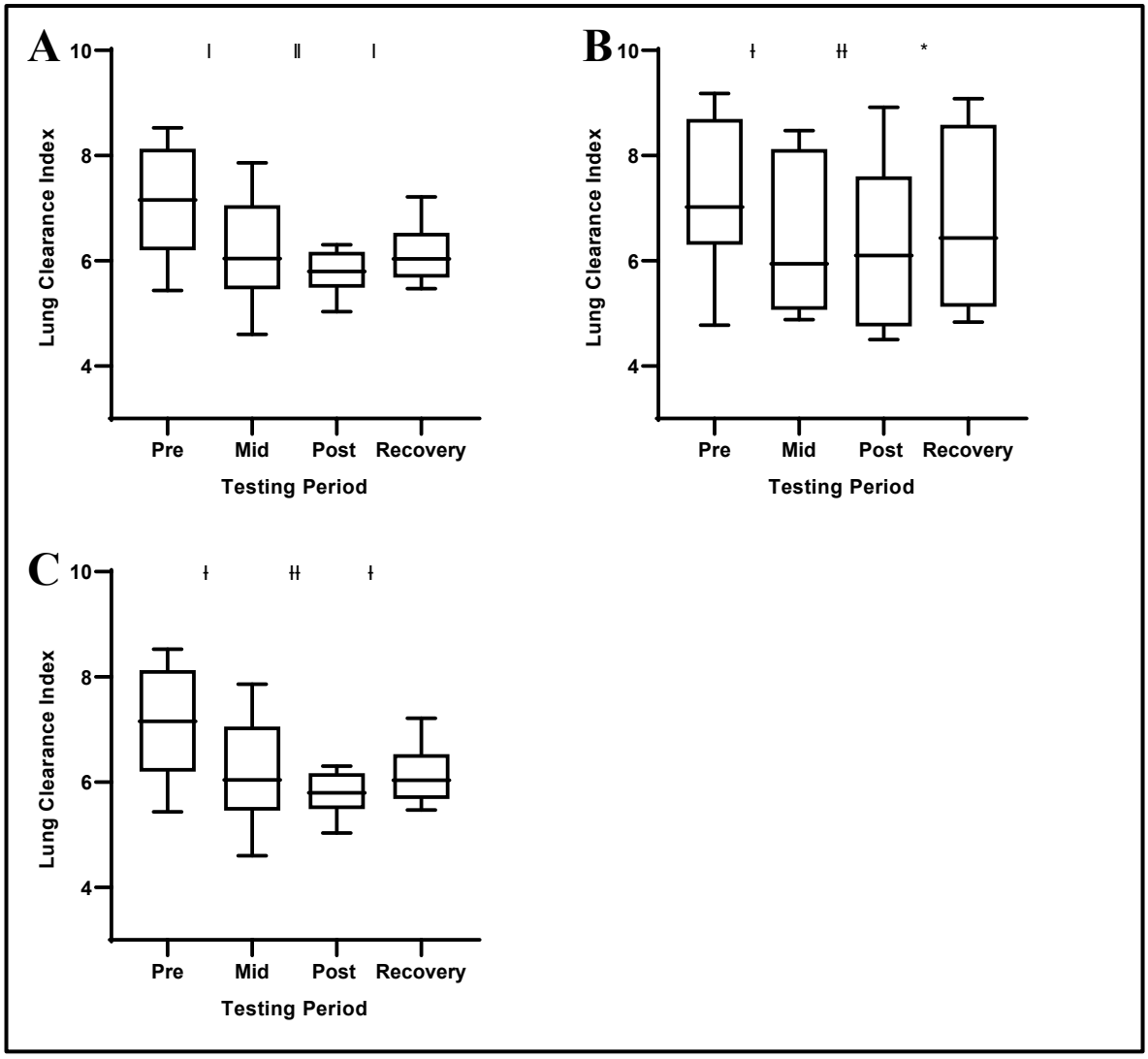


Values represent means ± SD. Ɨ Significant difference from the Pretesting period, $P < 0.01$.

4.3.2 Lung Clearance Index

For all oscillation exposures (i.e., 30s, 60s, and 120s), our cohort demonstrated a systematic reduction (i.e., improvement) in LCI from baseline testing, wherein LCI at the mid, post, and recovery testing periods were significantly better when compared with baseline LCI (**Table 10; Figure 11)**. This may be a result of distal alveolar atelectasis. Traditionally, LCI is considered a sensitive marker for air trapping in that lung units that receive less ventilation will take longer to clear the inert gas, leading to a higher (worse) LCI [47]. This interpretation of LCI is predicated on the lung units distal from an obstruction are still capable of being ventilated. However, it is at least conceivable that this relationship between air trapping and LCI may not be as well defined in a lung with a complete obstruction of ventilatory units. As such, a drop off in alveolar units (i.e., complete collapse and/or obstruction) in series may "improve" LCI. This could be due to a lower operational lung volume and less air trapping via fewer lung units capable of ventilation, allowing the inert gas (N_2 in this case) to be cleared with less expired gas.

Figure 11. Lung clearance index during the 30s, 60s, and 120s oscillation exposures.



Values represent means ± SD. **Panel A** depicts the 30s oscillations; **Panel B** depicts the 60s oscillations; and **Panel C** depicts the 120s oscillations. * Significant difference from the Pretesting period, $P < 0.05$. Ɨ Significant difference from the Pretesting period, $P < 0.01$. Ħ Significant difference from the Pretesting period, $P < 0.001$.

Table 10. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on lung clearance index.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	6.55	0.60	
Mid	5.89	0.58	**<0.01**
Post	5.84	0.78	**<0.001**
Recovery	5.96	0.67	**<0.01**
60s Oscillation Rate			
Pre	6.28	0.78	
Mid	5.58	0.59	**<0.01**
Post	5.39	0.69	**<0.001**
Recovery	5.62	0.62	**<0.05**
120s Oscillation Rate			
Pre	7.10	1.11	
Mid	6.14	1.03	**<0.01**
Post	5.78	0.44	**<0.001**
Recovery	6.15	0.57	**<0.01**

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

4.3.3 Arterial Blood Gases

In the 30s oscillation exposures, Hb and Hct were significantly higher at the mid and recovery testing periods when compared with the pretesting period (**Table 11; Table 12**). An elevated Hb and Hct may be indicative of an acute lung injury [48]. Indeed, cell-free hemoglobin has been shown to lead to oxidative stress, loss of nitric oxide, activation of inflammatory pathways [49-51]. This data supports our other findings in that we did observe possible alveolar atelectasis (diminished lung volumes and LCI) and pulmonary vascular vasoconstriction. Additional explanations for increases Hb and Hct may be hydration status. The subjects were asked to breath low humidity air (i.e., medical gases) for 45 continuous minutes during each exposure. Thus, there may have been a risk of dehydration. As such, we are unable to confidently attribute this change in Hct to environmental or physiologic changes. Additionally, while our data demonstrated no statistical difference in pH, RQ, VD/VT, A-a difference, or PaO_2 between any of the measurement time points there are non-significant trends of note. There was a trend towards a reduced A-a difference (**Table 13; Figure 12**), suggesting slight alveolar-capillary membrane thinning; an observation which aligns with the mild increases in Dm/Vc. Furthermore, there was a trend towards increasing PaO_2 and decreasing $PaCO_2$. This is not surprising given the length of exposure to high inspire O_2 concentrations. While there were no significant differences in VD/VT, there was an observed trend for slight increases (**Table 16; Figure 15**). An increased dead space volume may be evidence of mild atelectasis and/or pulmonary vascular derecruitment [52, 53]. These data suggest that exposure to 30s of oscillating inspired O_2/N_2 concentrations resulted in an increase in PaO_2 and decreased $PaCO_2$, possibly causing a downstream inflammatory response. However, any inflammatory response was not sufficient only to engender a very mild reduction in A-a difference.

In response to the 60s oscillations, our data demonstrated no statistically significant differences between any of the testing periods for pH, RQ, PaO2, VD/VT, or A-a difference. We did observe significantly higher Hb at the recovery testing period and Hct at the post and recovery periods compared with the pretesting period (**Table 11; Table 12**). This elevation of Hb and Hct may suggest the presence of acute lung injury (see above). An interesting aspect of this finding is the delayed nature of the presentation. This suggests there may be an additive effect of exposure 1 and 2 and that there may be additional delayed physiologic changes as a result of this exposure. Additionally, the 60s oscillation data demonstrated a similar trend in $PaCO_2$ as the 30s oscillations in that there was a trend towards decreasing $PaCO_2$ with a significantly lower $PaCO_2$ at the post and recovery testing period (**Table 15; Figure 14**). Unlike the 30s oscillation exposure, we observed a trend towards an increased A-a difference in response to 60s oscillations (**Table 13; Figure 12**). This increase A-a difference may suggest slight alveolar-capillary membrane thickening. These data suggest exposure to the 60s oscillations may upregulate inflammatory pathways and induce oxidative stress. However, it is important to note that these changes may be delayed in their presentation, suggesting a need for longer term, successive testing post exposure.

In response to the 120s oscillations, we observed no differences in pH, Hb, Hct, RQ, VD/VT, A-a difference, or PaO_2. Our data did show a significantly decreased PaCO2 at the recovery period when compared with the pretesting period and a tendency for a rise in PaO_2 (**Table 14; Table 15; Figure 13; Figure 14)**. We also observed similar trends in A-a difference and VD/VT as the 30s oscillations. Specifically, there was a trend for a slight fall in A-a difference, suggesting mild alveolar-capillary membrane thinning (**Table 13; Figure 12**). Additionally, there was a trend for an increase in VD/VT, suggesting possible mild atelectasis and/or pulmonary vascular derecruitment (**Table 16; Figure 15)** [52, 53].

Table 11. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on hemoglobin.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	14.04	1.62	
Mid	14.48	1.59	**<0.05**
Post	14.39	1.45	0.09
Recovery	14.51	1.55	**<0.05**
60s Oscillation Rate			
Pre	14.11	0.99	
Mid	14.40	1.00	0.09
Post	14.41	1.08	0.08
Recovery	14.51	0.92	**<0.01**
120s Oscillation Rate			
Pre	14.04	0.79	
Mid	14.38	0.99	0.51
Post	14.36	1.01	0.54
Recovery	14.26	1.13	0.79

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 12. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on hematocrit.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	41.69	4.57	
Mid	42.58	4.74	**<0.05**
Post	42.31	4.27	0.08
Recovery	42.69	4.53	**<0.05**
60s Oscillation Rate			
Pre	41.50	2.94	
Mid	42.33	3.00	0.08
Post	42.43	3.22	**<0.05**
Recovery	42.71	2.75	**<0.01**
120s Oscillation Rate			
Pre	41.31	2.42	
Mid	42.25	2.92	0.53
Post	42.25	2.96	0.53
Recovery	42.13	3.35	0.64

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 13. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on arterial-alveolar oxygen difference.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	1.04	7.04	
Mid	1.49	9.69	0.99
Post	–2.18	5.09	0.58
Recovery	–2.71	4.99	0.45
60s Oscillation Rate			
Pre	0.16	4.18	
Mid	–2.17	3.29	0.59
Post	–1.52	6.42	0.79
Recovery	–0.03	8.03	0.99
120s Oscillation Rate			
Pre	2.44	4.84	
Mid	–0.08	3.55	0.69
Post	–1.50	4.06	0.34
Recovery	1.64	6.21	0.98

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 14. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on arterial partial pressure of oxygen.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	98.69	15.96	
Mid	104.88	12.67	0.39
Post	102.35	9.88	0.77
Recovery	104.94	17.07	0.38
60s Oscillation Rate			
Pre	113.29	14.78	
Mid	115.93	11.15	0.78
Post	115.71	13.19	0.82
Recovery	114.36	14.72	0.98
120s Oscillation Rate			
Pre	101.13	7.73	
Mid	103.81	9.13	0.94
Post	107.31	13.39	0.57
Recovery	108.19	17.44	0.46

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 15. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on arterial partial pressure of carbon dioxide.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	37.51	4.44	
Mid	35.43	7.28	0.31
Post	36.66	5.35	0.89
Recovery	34.08	7.14	**<0.05**
60s Oscillation Rate			
Pre	31.5	8.51	
Mid	29.97	7.36	0.43
Post	28.34	8.15	**<0.05**
Recovery	28.47	8.39	**<0.05**
120s Oscillation Rate			
Pre	36.68	4.43	
Mid	35.56	4.84	0.88
Post	33.91	7.37	0.29
Recovery	30.48	5.99	**<0.01**

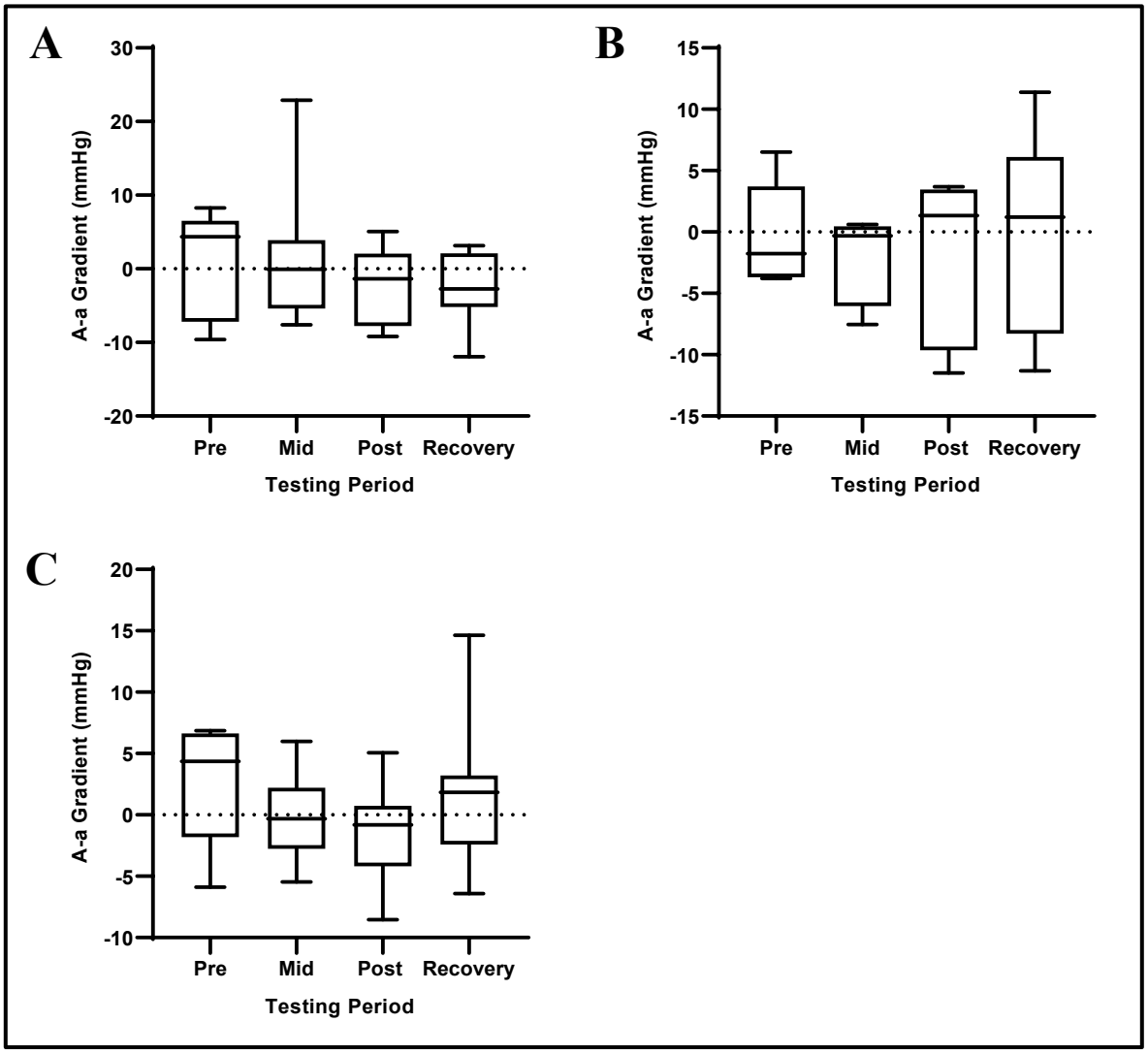
SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Table 16. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on the ratio of dead space to tidal volume.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	0.21	0.05	
Mid	0.21	0.04	0.99
Post	0.23	0.05	0.42
Recovery	0.22	0.04	0.81
60s Oscillation Rate			
Pre	0.17	0.05	
Mid	.020	0.07	0.22
Post	0.17	0.07	0.99
Recovery	0.16	0.07	0.99
120s Oscillation Rate			
Pre	0.19	0.04	
Mid	0.21	0.04	0.39
Post	0.21	0.04	0.53
Recovery	0.20	0.03	0.78

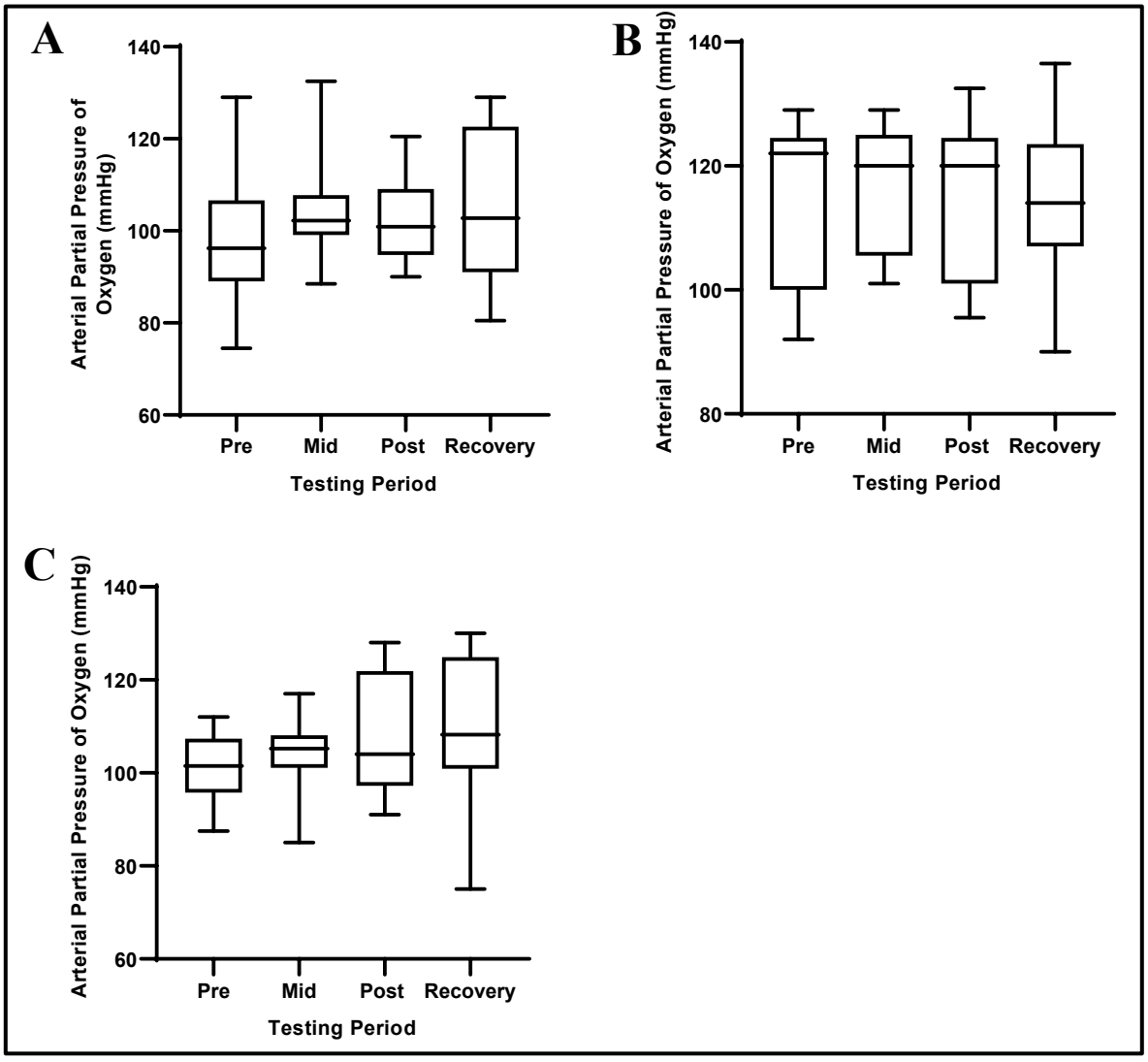
SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Figure 12. Arterial-alveolar oxygen difference during the 30s, 60s, and 120s oscillation exposures.



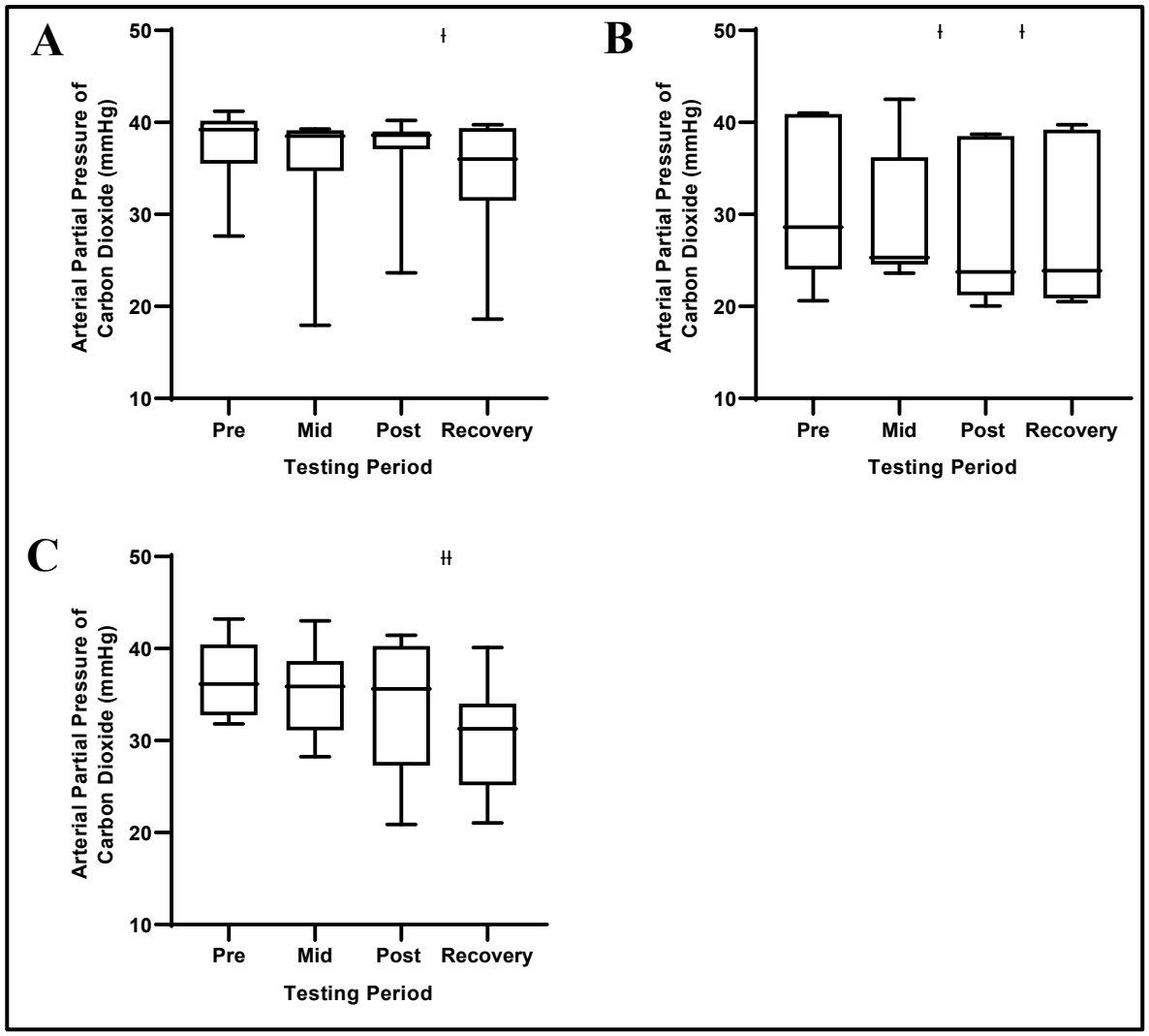
Values represent means ± SD. **Panel A** depicts the 30s oscillations; **Panel B** depicts the 60s oscillations; and **Panel C** depicts the 120s oscillations. There were no significant differences

Figure 13. Arterial partial pressure of oxygen during the 30s, 60s, and 120s oscillation exposures.



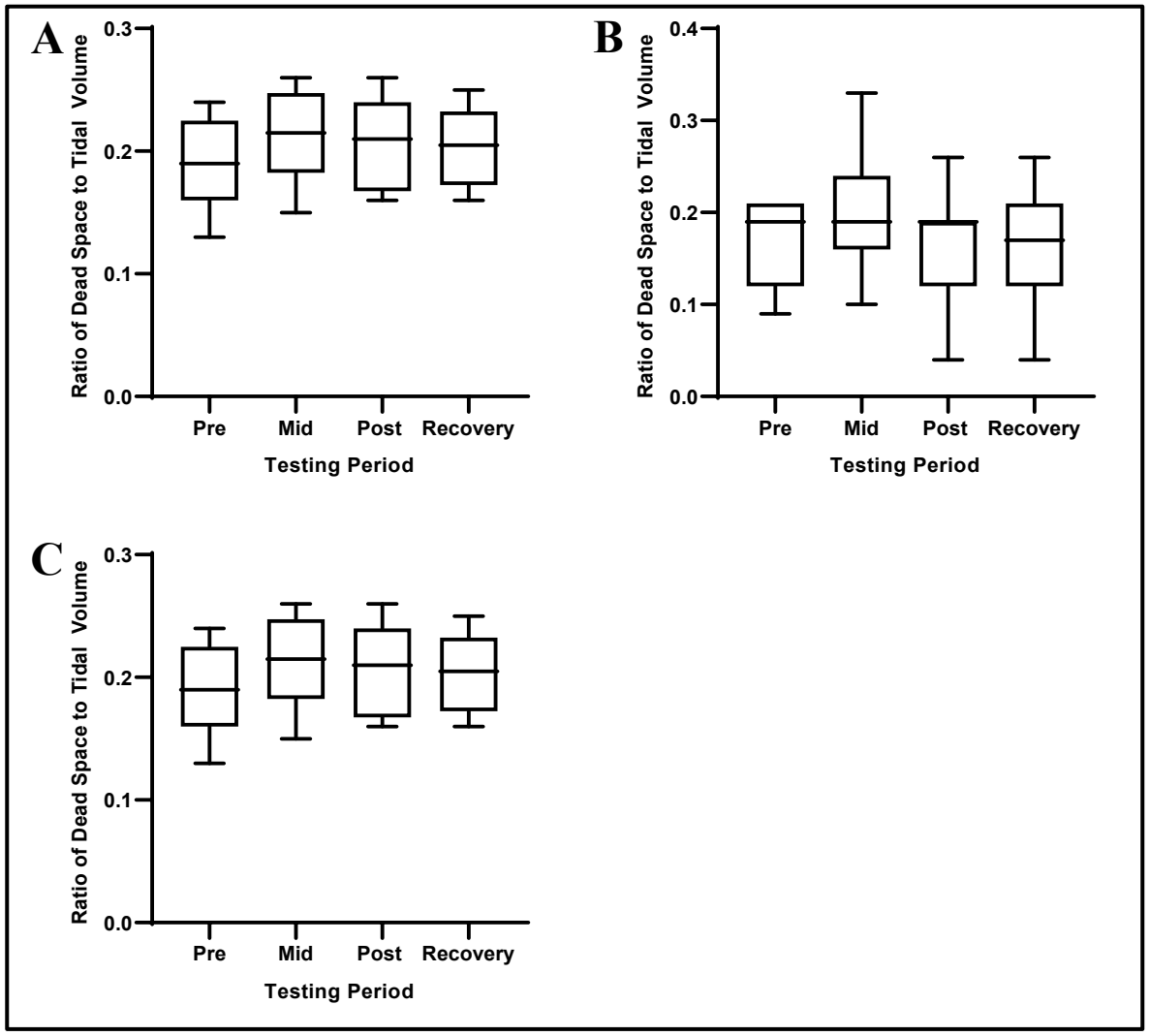
Values represent means ± SD. **Panel A** depicts the 30s oscillations; **Panel B** depicts the 60s oscillations; and **Panel C** depicts the 120s oscillations. There were no significant differences.

Figure 14. Arterial partial pressure of carbon dioxide during the 30s, 60s, and 120s oscillation exposures.



Values represent means ± SD. **Panel A** depicts the 30s oscillations; **Panel B** depicts the 60s oscillations; and **Panel C** depicts the 120s oscillations. ǂ Significant difference from the Pretesting period, $P < 0.05$. ǂǂ Significant difference from the Pretesting period, $P < 0.01$.

Figure 15. The ratio of dead space to tidal volume during the 30s, 60s, and 120s oscillation exposures.



Values represent means ± SD. **Panel A** depicts the 30s oscillations; **Panel B** depicts the 60s oscillations; and **Panel C** depicts the 120s oscillations. There were no significant differences.

4.4 Hemodynamics

There were no changes in Q observed in response to the 60s or 120s oscillations at any testing period. However, we did demonstrate a significant reduction in Q at the post-testing period when compared with the pretesting period in response to the 30s oscillations, a reduction that was not maintained at the recovery period (**Table 17**). This is not surprising as hyperoxia has been demonstrated to blunt Q [54]. These data suggest that exposure to shorter O_2/N_2 oscillations may have a more pronounced effect on Q compared with longer exposures.

Table 17. The influence of 30s, 60s. and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on cardiac output.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	5.48	1.02	
Mid	5.16	0.98	0.34
Post	4.88	0.83	**<0.05**
Recovery	5.01	0.97	0.11
60s Oscillation Rate			
Pre	5.39	1.08	
Mid	5.09	1.07	0.69
Post	5.45	1.29	0.99
Recovery	5.29	1.32	0.98
120s Oscillation Rate			
Pre	5.52	1.04	
Mid	5.31	0.77	0.80
Post	5.31	0.78	0.80
Recovery	5.37	0.93	0.91

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

4.4.1 Nonlinear Trends During the 30s Oscillation Exposure

Table 18 and Figure 16 illustrates the effect of time on systolic BP during the 30s oscillation exposure. We observed no changes in impedance (44.82 ± 13.66 Ohms). There was a systematic reduction in BP during Arm 1 and an increase in BP during Arm 2. Furthermore, HR demonstrates a sinusoidal relationship with time wherein HR rises during the initial 20min of both exposure Arms with a regression towards baseline at the completion of Arm 1 and Arm 2 (**Table 19; Figure 17**). We also observed a similar relationship in the influence of exposure time on RSO_2 (**Table 22; Figure 19**). Specifically, RSO_2 rose rapidly in the first 10min of Arm 1 with a regression to baseline at the end of Arm 1. On the other hand, RSO_2 increased for the duration of Arm 2. Conversely, CBF demonstrated a non-linear, quadratic relationship with time, in that CBF was observed to fall slightly during study Arm 1 with a rise towards baseline during Arm 2 (**Table 21; Figure 18**). Our data demonstrated a negative relationship between $P_{ET}CO_2$ and exposure length during both exposure Arms (**Table 20; Figure 20**).

We also observed a positive relationship between $P_{ET}CO_2$ and BP and RSO_2 where a drop in $P_{ET}CO_2$ is associated with decreases in both BP and RSO_2. Conversely, there was a negative relationship between $P_{ET}CO_2$ and HR. These findings support previous literature insofar as hypocapnia has been shown to increase cardiovascular parameters through sympathetic activity [55]. Additionally, exposure to acute altitude has also been shown to increase these parameters through activation of the peripheral chemoreceptors and downstream sympathetic upregulation [56]. Further, hypocapnia may decrease BP via depression of the central chemoreceptors, resulting in a secondary reflex reduction of the arterial baroreceptors to induce a compensatory increase in

HR [57]. However, we did observe an increase in BP during exposure Arm 2. This may be partially explained by the direct vasoconstrictive effect of hyperoxia or the increased sympathetic drive associated with altitude exposure, increasing BP [56, 58]. This suggests prolonged exposure to hyperoxia, particularly in the background of mild altitude, may result in the vasoconstriction we observed in the 30s oscillation exposure. Additionally, there is an established negative feedback baroreflex mechanism in which BP and HR change in opposite directions, as seen in the current study [59-61]. Furthermore, our data demonstrated a positive relationship between CBF and BP and $P_{ET}CO_2$, but a negative relationship with HR. Specifically, as CBF rises, both BP and $P_{ET}CO_2$ increase in tandem while HR decreases. These data are not surprising as previous literature suggests a dynamic relationship between CBF and $P_{ET}CO_2$ wherein hypocapnia may decrease CBF [62, 63]. Additionally, any deviation from ~70-80% RSO_2 is associated with a decrease in BP and CBF. Taken together, these data suggest a dynamic relationship between $P_{ET}CO_2$ and cardiovascular parameters through modulation of the central chemoreceptors. Specifically, the hypocapnia induced by exposure to the 30s oscillations may inhibit the central chemoreceptors, resulting in downstream decrease in BP and a compensatory increase in HR.

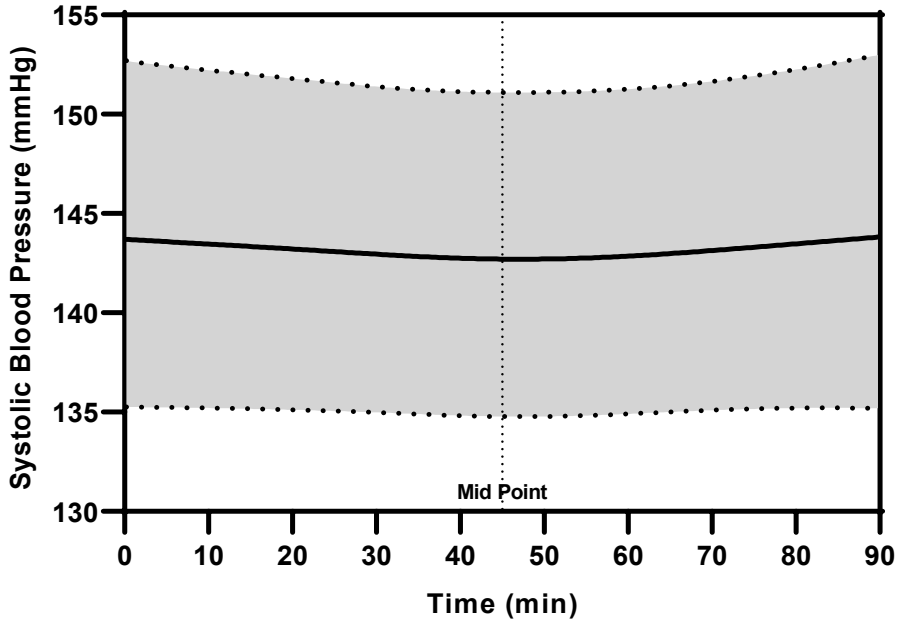


Figure 16. The effect of exposure length on systolic blood pressure during the 30s oscillation rate

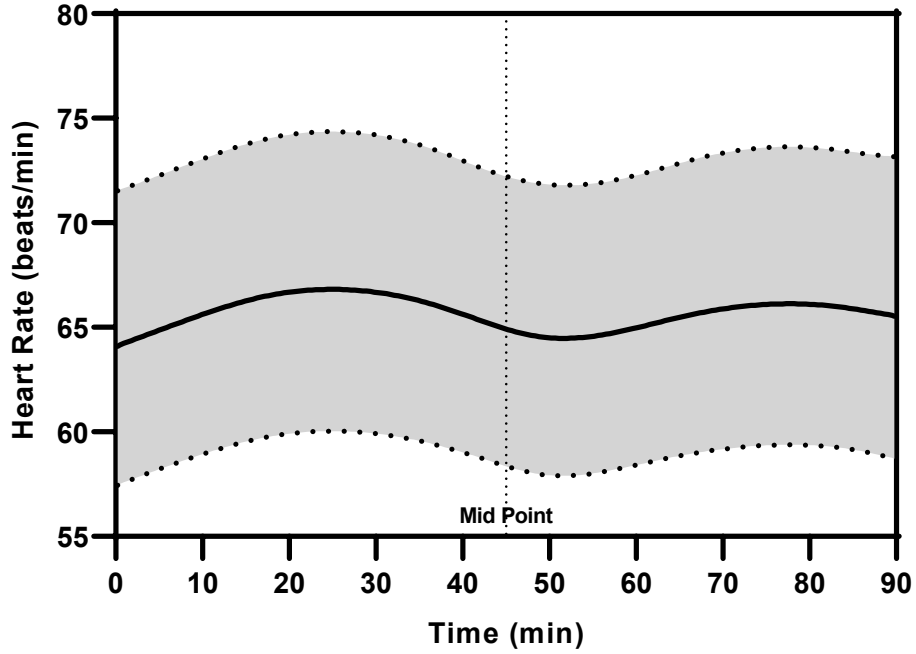


Figure 17. The effect of exposure length on heart rate during the 30s oscillation rate

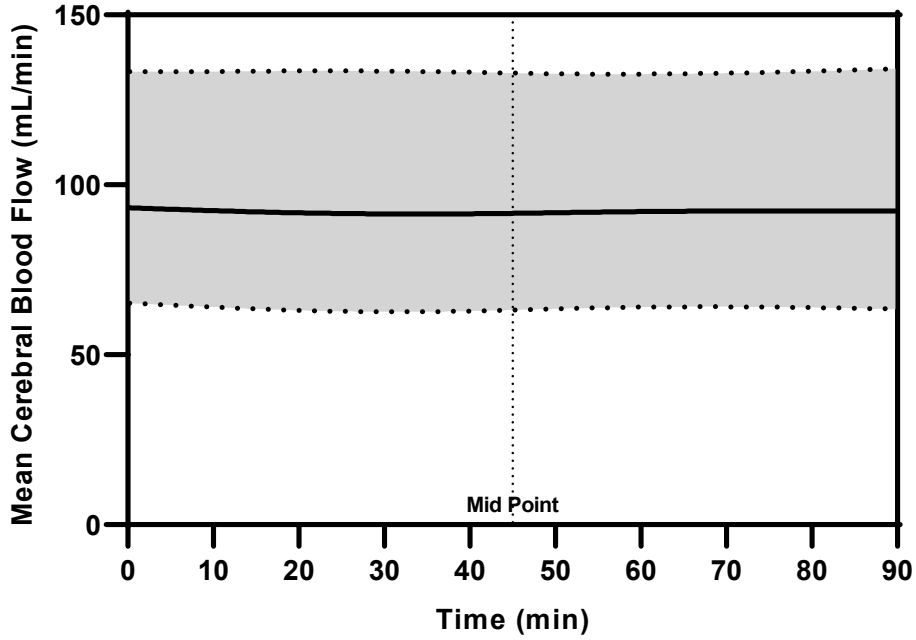


Figure 18. The effect of exposure length on mean cerebral blood flow during the 30s oscillation rate

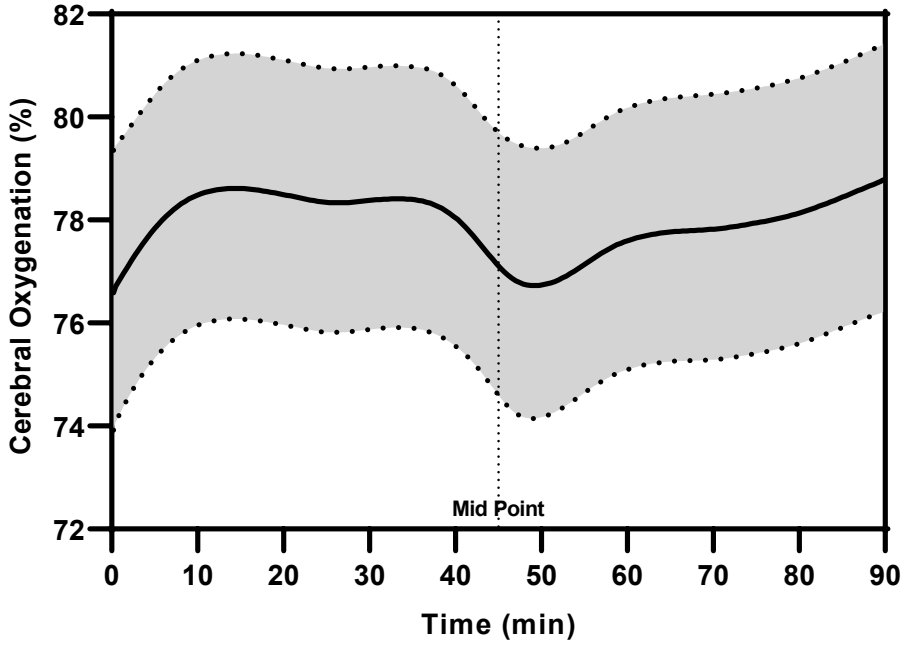


Figure 19. The effect of exposure length on cerebral oxygenation during the 30s oscillation rate

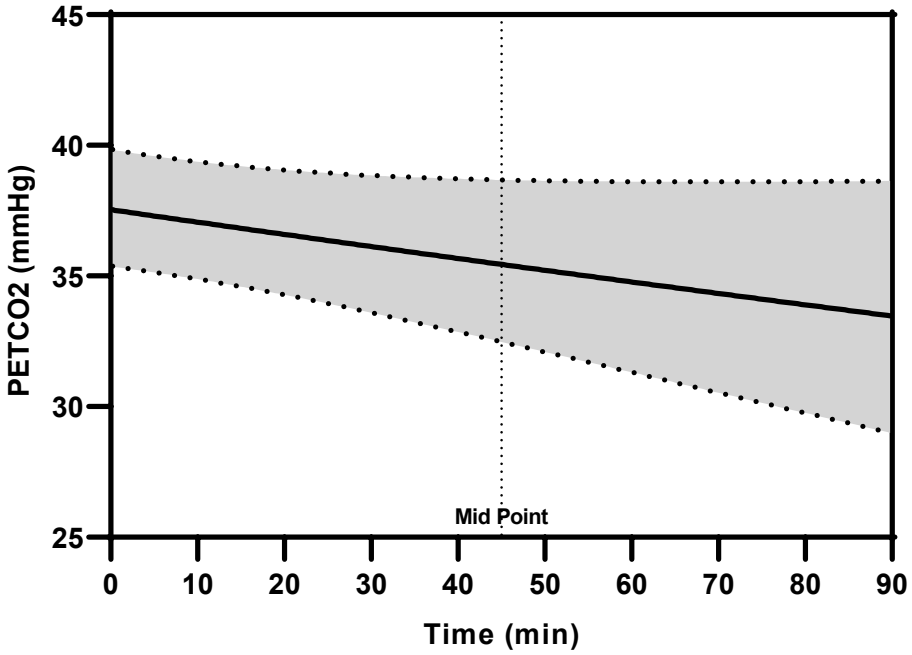


Figure 20. The effect of exposure length on PETCO2 during the 30s oscillation rate

Table 18. GAMM results for blood pressure during the 30s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	5.02	0.02	281.30	**<0.001**
s(Time)	0.67	-	0.52	**<0.01**
s(Heart Rate)	<0.01	-	0.00	0.08
s(Impedance)	5.55	-	13291.50	**<0.001**
s(Cerebral Oxygenation)	1.10	-	4.71	**<0.001**
s(PETCO2)	0.60	-	4.64	**<0.001**
s(Cerebral Blood Flow)	<0.01	-	0.00	**<0.05**
Random Effects				
ID	<0.01	-	0.02	**<0.01**
ID:Time	8.78	-	417.33	**<0.001**
ID:Heart Rate	<0.01	-	0.00	0.05
ID:Impedance	<0.01	-	0.00	0.09
ID:Cerebral Oxygenation	6.36	-	2440.73	**<0.001**
ID:PETCO2	<0.01	-	0.00	**<0.001**
ID:Cerebral Blood Flow	<0.01	-	0.00	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; Impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral

blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on blood pressure (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 19. GAMM results for heart rate during the 30s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.20	0.03	136.5	**<0.001**
s(Time)	3.43	-	4.83	**<0.001**
s(Blood Pressure)	<0.01	-	0.00	0.19
s(Impedance)	0.66	-	1970.25	**<0.001**
s(Cerebral Oxygenation)	<0.01	-	0.00	0.46
s(PETCO2)	0.56	-	80.28	**<0.001**
s(Cerebral Blood Flow)	0.72	-	270.21	**<0.001**
Random Effects				**<0.001**
ID	<0.01	-	0.00	**<0.001**
ID:Time	6.03	-	16644.91	**<0.001**
ID:Blood Pressure	3.09	-	30148.27	**<0.001**
ID:Impedance	<0.01	-	0.00	**<0.01**
ID:Cerebral Oxygenation	3.71	-	46727.59	**<0.001**
ID:PETCO2	<0.01	-	0.04	**<0.001**
ID:Cerebral Blood Flow	4.38	-	37437.37	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; blood pressure was measure in mmHg; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on heart rate (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 20. GAMM results for end-tidal CO_2 during the 30s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	1.81	0.02	75.26	**<0.001**
s(Time)	0.77	-	2742.45	**<0.001**
s(Heart Rate)	0.15	-	1.49	**<0.05**
s(Blood Pressure)	0.44	-	15.99	**<0.01**
s(Impedance)	0.91	-	18328.76	**<0.001**
s(Cerebral Oxygenation)	0.79	-	328.07	**<0.001**
s(Cerebral Blood Flow)	5.34	-	9070.36	**<0.001**
Random Effects				
ID	2.50	-	274.01	0.42
ID:Time	7.47	-	388.71	**<0.001**
ID:Heart Rate	<0.01	-	0.00	**<0.001**
ID:Blood Pressure	<0.01	-	0.00	**<0.001**
ID:Impedance	3.85	-	1689.75	**<0.05**
ID:Cerebral Oxygenation	<0.01	-	0.00	**<0.001**
ID:Cerebral Blood Flow	2.59	-	1083.41	**<0.01**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on end-tidal CO_2 (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 21. GAMM results for mean cerebral blood flow during the 30s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.48	0.12	37.64	**<0.001**
s(Time)	1.13	-	0.56	**<0.001**
s(Heart Rate)	1.44	-	38.69	**<0.001**
s(Blood Pressure)	<0.01	-	0.00	0.06
s(Impedance)	<0.01	-	0.00	0.11
s(PETCO2)	2.25	-	1938.05	**<0.001**
s(Cerebral Oxygenation)	1.46	-	8.69	**<0.001**
Random Effects				
ID	1.69	-	10033.64	**<0.001**
ID:Time	6.30	-	397.83	**<0.001**
ID:Heart Rate	<0.01	-	0.00	**<0.001**
ID:Blood Pressure	<0.01	-	0.00	**<0.01**
ID:Impedance	4.52	-	35961.45	**<0.001**
ID:PETCO2	<0.01	-	0.00	**<0.001**
ID:Cerebral Oxygenation	5.83	-	57531.98	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; PETCO2 was measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on mean cerebral blood flow (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 22. GAMM results for cerebral oxygenation during the 30s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.33	0.01	355.8	**<0.001**
s(Time)	7.73	-	11.96	**<0.001**
s(Heart Rate)	<0.01	-	0.00	0.12
s(Blood Pressure)	<0.01	-	0.00	0.31
s(Impedance)	4.96	-	18818.12	**<0.001**
s(PETCO2)	3.36	-	194.72	**<0.001**
s(Cerebral Blood Flow)	<0.01	-	0.00	0.07
Random Effects				
ID	5.24	-	7328.10	**<0.001**
ID:Time	5.26	-	451.00	**<0.001**
ID:Heart Rate	4.06	-	4939.61	**<0.001**
ID:Blood Pressure	<0.01	-	0.00	**<0.05**
ID:Impedance	<0.01	-	0.00	**<0.05**
ID:PETCO2	<0.01	-	0.00	**<0.05**
ID:Cerebral Blood Flow	5.69	-	28466.38	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on cerebral oxygenation (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

4.4.2 Nonlinear Trends During the 60s Oscillation Exposure

In response to the 60s oscillation rate, HR rose during the first 20min of both exposure Arms with a regression towards resting HR at the end of Arm 1 and 2 (**Table 23; Figure 21**). We observed a similar trend in $P_{ET}CO_2$ across both exposure Arms during the 60s oscillations as were observed in the 30s oscillation rate with comparable magnitudes (-4.1 and -3.9mmHg for the 30s and 60s oscillations respectively) (**Table 24; Figure 22**). Again, there were similar trends in the influence of time on RSO_2 in the 60s oscillation exposure and the 30s oscillations. Specifically, RSO_2 increased during the initial 15-20min of both exposure Arms with a regression towards baseline at the end of the exposure Arm (**Table 26; Figure 24**). On the other hand, we observed a significant, albeit very mild, increase in CBF across time during both study Arms (**Table 25; Figure 23**). Additionally, exposure time had a significant influence on thoracic impedance wherein changes in impedance exhibit a sinusoidal trend (**Table 27; Figure 25**).

Interestingly, we observed an inverse relationship between $P_{ET}CO_2$ and HR, systolic BP, and thoracic impedance; simply, as $P_{ET}CO_2$ decreases, HR, BP, and impedance increase. These data are not surprising as previous literature suggests hypocapnia and acute altitude exposure induce an increase in HR through sympathetic activation (see above) [55, 56]. Interestingly, we did not observe a change in BP in response to the 60s oscillation exposure. This may be due to hyperoxia-induced deactivation of the carotid body chemoreceptors, inducing competing reduction in sympathetic activity, maintaining BP [58, 64, 65]. Therefore, the increased exposure to hyperoxia (60s vs 30s oscillations) may modulate the influence of hypocapnia on the sympathetic nervous system, explaining the increase in HR and the maintenance of BP. We also observed similar relationships between CBF and $P_{ET}CO_2$ as those demonstrated during the 30s oscillation groups in that as CBF rises, as does $P_{ET}CO_2$. Furthermore, any deviation from ~70-80% RSO_2 is associated

with a decrease in CBF. Additionally, as $P_{ET}CO_2$ decreases, RSO_2 decreases as well. These findings are support by previous literature wherein hypocapnia has been shown to reduce cerebral oxygenation and CBF [62, 63, 66]. However, we observed an increase in RSO_2 in response to the 60s hyperoxia oscillations. As such, it appears the ability of hyperoxia to increase cerebral oxygenation [67] exerted a stronger influence on cerebral oxygenation than does hypocapnia in the present study.

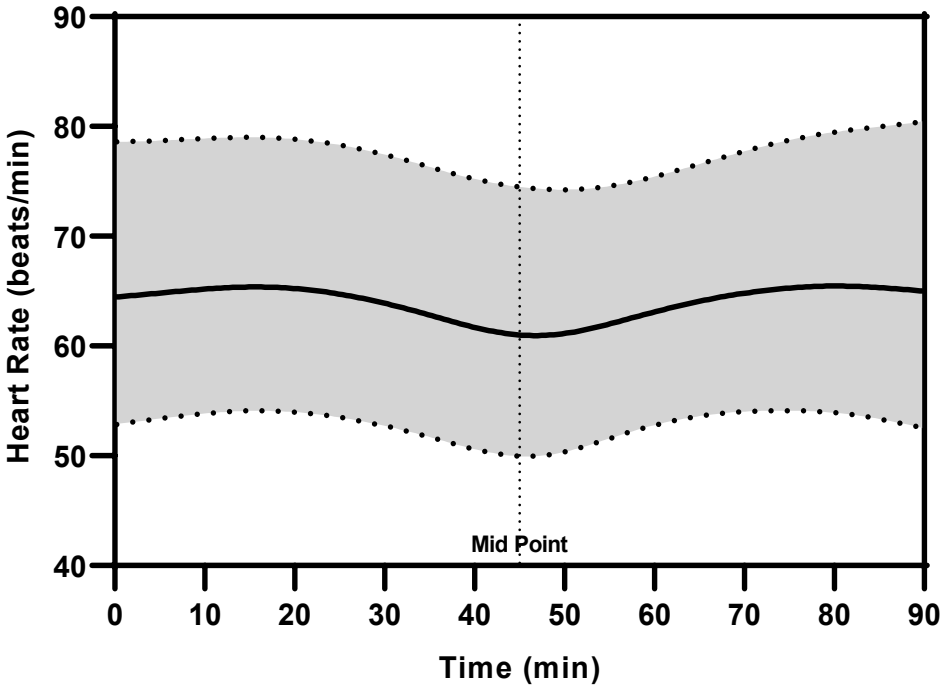


Figure 21. The effect of exposure length on heart rate during the 60s oscillation rate

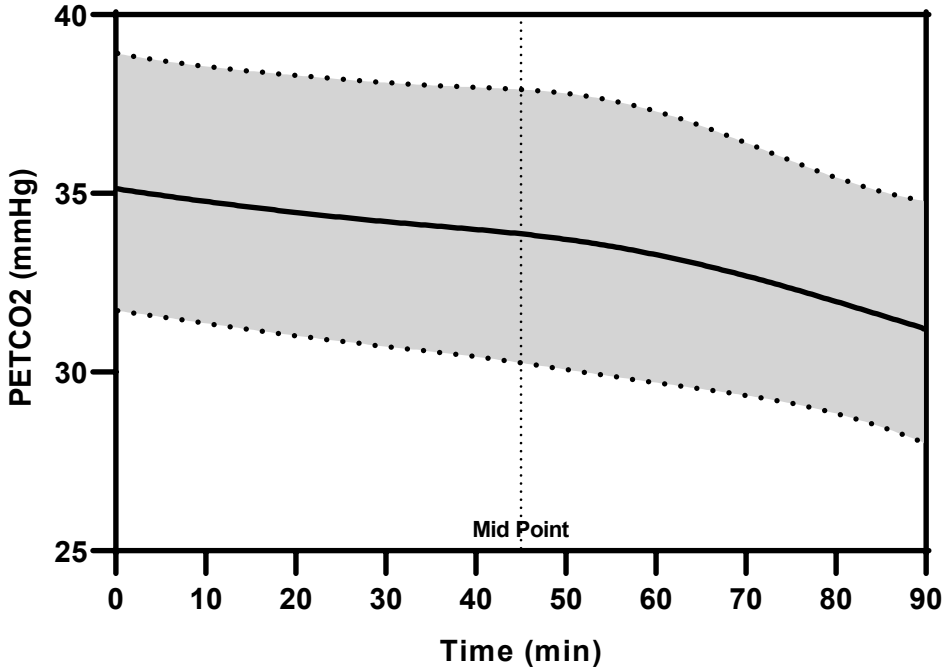


Figure 22. The effect of exposure length on PETCO2 during the 60s oscillation rate

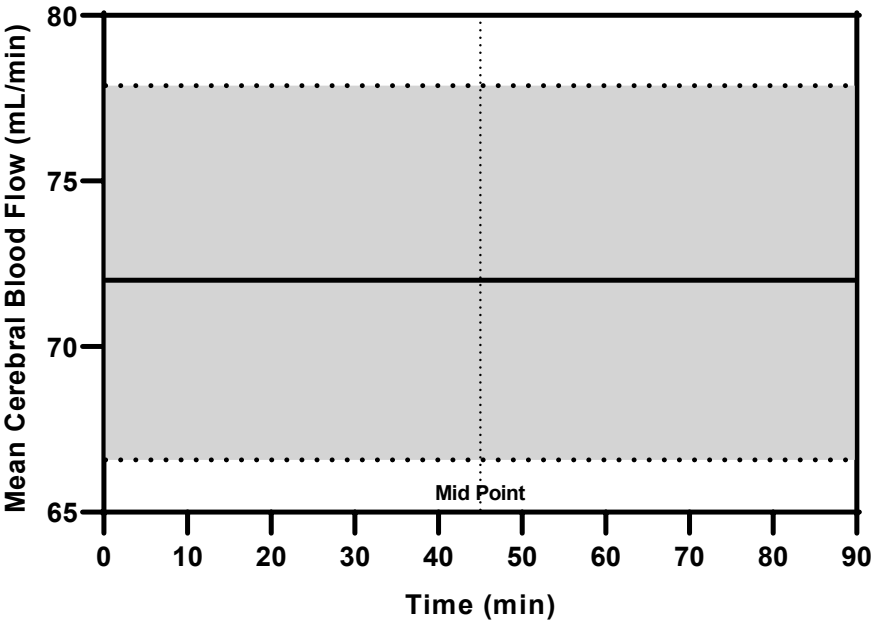


Figure 23. The effect of exposure length on mean cerebral blood flow during the 60s oscillation rate.

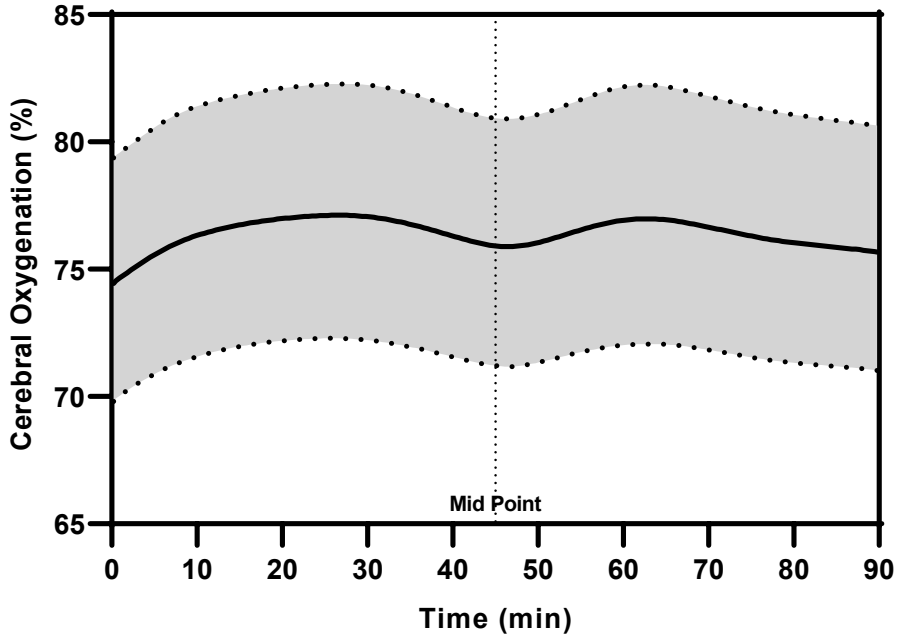


Figure 24. The effect of exposure length on cerebral oxygenation during the 60s oscillation rate

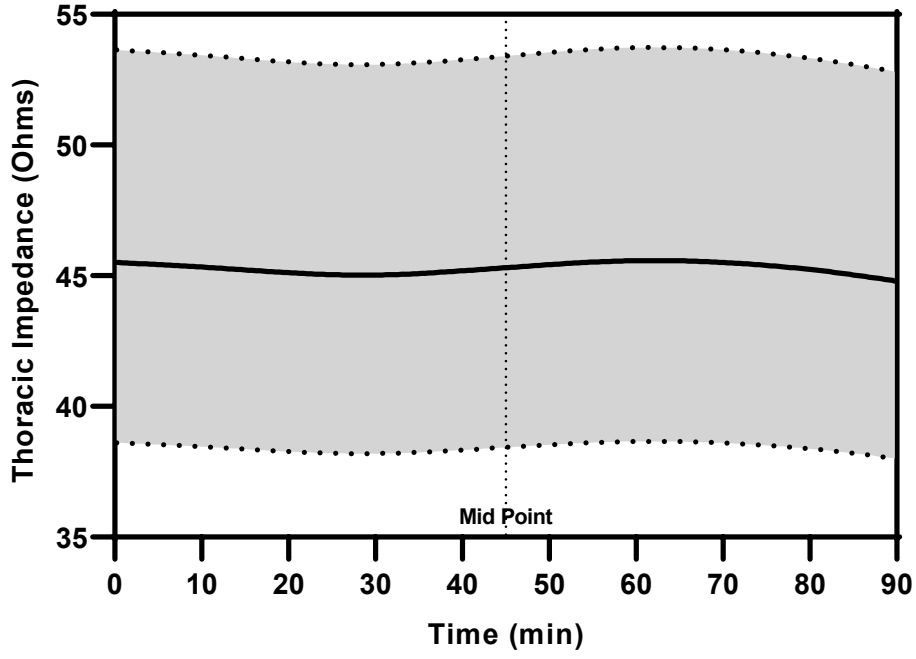


Figure 25. The effect of exposure length on thoracic impedance during the 60s oscillation rate

Table 23. GAMM results for heart rate during the 60s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.17	0.04	97.6	**<0.001**
s(Time)	3.58	-	14.59	**<0.001**
s(Blood Pressure)	<0.01	-	0.00	**<0.05**
s(Impedance)	<0.01	-	0.00	0.23
s(Cerebral Oxygenation)	0.55	-	82.97	**<0.001**
s(PETCO2)	0.86	-	703.65	**<0.001**
s(Cerebral Blood Flow)	<0.01	-	0.00	0.38
Random Effects				
ID	0.16	-	4.07	0.61
ID:Time	<0.01	-	0.00	**<0.05**
ID:Blood Pressure	<0.01	-	0.00	**<0.001**
ID:Impedance	<0.01	-	0.00	0.10
ID:Cerebral Oxygenation	3.88	-	537.49	**<0.001**
ID:PETCO2	<0.01	-	0.01	**<0.01**
ID:Cerebral Blood Flow	<0.01	-	0.00	0.05

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; blood pressure was measure in mmHg; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on heart rate (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 24. GAMM results for end-tidal CO_2 during the 60s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	1.78	0.03	65.44	**<0.001**
s(Time)	2.22	-	6.59	**<0.001**
s(Heart Rate)	0.86	-	38.05	**<0.001**
s(Blood Pressure)	0.79	-	2.09	**<0.001**
s(Impedance)	<0.01	-	0.00	0.12
s(Cerebral Oxygenation)	3.17	-	11.03	0.19
s(Cerebral Blood Flow)	0.97	-	39.31	**<0.001**
Random Effects				
ID	2.99	-	235.12	**<0.001**
ID:Time	0.31	-	0.41	**<0.001**
ID:Heart Rate	1.29	-	10.00	**<0.05**
ID:Blood Pressure	<0.01	-	0.00	**<0.05**
ID:Impedance	<0.01	-	0.00	**<0.05**
ID:Cerebral Oxygenation	<0.01	-	0.00	**<0.01**
ID:Cerebral Blood Flow	<0.01	-	0.00	**<0.01**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on end-tidal CO_2 (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 25. GAMM results for mean cerebral blood flow during the 60s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.27	0.03	139.70	**<0.001**
s(Time)	<0.01	-	0.00	**<0.01**
s(Heart Rate)	0.31	-	0.24	**<0.05**
s(Blood Pressure)	<0.01	-	0.00	0.11
s(Impedance)	<0.01	-	0.00	0.09
s(PETCO2)	0.97	-	680.49	**<0.001**
s(Cerebral Oxygenation)	<0.01	-	0.00	0.17
Random Effects				
ID	<0.01	-	0.00	**<0.05**
ID:Time	2.39	-	65.50	**<0.05**
ID:Heart Rate	<0.01	-	0.00	**<0.05**
ID:Blood Pressure	<0.01	-	0.00	0.07
ID:Impedance	<0.01	-	0.00	0.11
ID:PETCO2	3.91	-	1792.55	**<0.001**
ID:Cerebral Oxygenation	<0.01	-	0.00	**<0.01**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; PETCO2 was measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on mean cerebral blood flow (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 26. GAMM results for cerebral oxygenation during the 60s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.35	0.02	220.00	**<0.001**
s(Time)	5.30	-	7.69	**<0.001**
s(Heart Rate)	<0.01	-	0.00	0.26
s(Blood Pressure)	2.62	-	43.78	**<0.001**
s(Impedance)	0.85	-	978.72	**<0.001**
s(PETCO2)	0.91	-	416.55	**<0.001**
s(Cerebral Blood Flow)	0.82	-	0.76	**<0.001**
Random Effects				
ID	2.22	-	6911.55	**<0.001**
ID:Time	3.64	-	239.24	**<0.001**
ID:Heart Rate	1.58	-	341.12	**<0.001**
ID:Blood Pressure	<0.01	-	0.00	**<0.01**
ID:Impedance	<0.01	-	0.00	**<0.05**
ID:PETCO2	2.61	-	217.13	0.22
ID:Cerebral Blood Flow	1.82	-	180.87	**<0.05**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on cerebral oxygenation (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 27. GAMM results for thoracic impedance during the 60s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	3.82	0.08	49.22	**<0.001**
s(Time)	2.87	-	4.96	**<0.001**
s(Heart Rate)	<0.01	-	0.00	0.38
s(Blood Pressure)	<0.01	-	0.00	0.37
s(Cerebral Oxygenation)	0.55	-	<0.01	**<0.001**
s(PETCO2)	<0.01	-	0.00	**<0.05**
s(Cerebral Blood Flow)		-	2.39	**<0.01**
Random Effects				
ID	3.99	-	<0.01	**<0.001**
ID:Time	4.39	-	<0.01	**<0.001**
ID:Heart Rate	<0.01	-	0.00	**<0.05**
ID:Blood Pressure	<0.01	-	0.00	0.77
ID:Impedance	<0.01	-	0.00	0.21
ID:Cerebral Oxygenation	<0.01	-	0.00	0.22
ID:Cerebral Blood Flow	<0.01	-	<0.01	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on thoracic impedance (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

4.4.3 Nonlinear Trends During the 120s Oscillation Exposure

Table 28 and Figure 26 illustrates the influence of exposure time on BP during the 120s oscillations. There were no changes in CBF (72.46 ± 17.71 mL/min). We observed a progressive decrease in BP during exposure Arm 1 and the first ~15min of Arm 2, after which BP increased towards baseline at the end of Arm 2. During the 120s oscillations, we observed a similar trend in HR and RSO_2 as during the 30s and 60s oscillation exposures (**Table 29; Table 30; Figure 27; Figure 28**). Specifically, both HR and RSO_2 exhibited a sinusoidal trend in that these metrics rose during the initial ~20-25min of Arm 1 and Arm 2 with a regression towards baseline at the end of Arm 1 and Arm 2. Furthermore, our data demonstrated a systematic increase in thoracic impedance across both exposure Arms (**Table 31; Figure 29**). In contrast to the 30s and 60s oscillation exposures, we did not observe a significant difference in $P_{ET}CO_2$ or CBF in response to the 120s oscillations.

Our data also demonstrate various interactions between the above covariates of interest. Heart rate appears to have a modulating influence on various hemodynamic measures. Heart rates <55 and >100 beats/min are associated with increased BP. These data are not surprising as previous literature suggests exposure to hyperoxia may deactivate the carotid body chemoreceptors [58, 64, 65]. Furthermore, while our data do not suggest a significant change in $P_{ET}CO_2$, participants did exhibit clinical hypocapnia (31-35mmHg) [68]. The observed clinical hypocapnia demonstrated by the participants may reduce BP via depression of the central chemoreceptors and a reflex reduction of the arterial baroreceptors to engender a compensatory increase in HR [57]. Interestingly, we observed an increase in BP towards baseline at the end of exposure Arm 2, which may be partially explained by the direct vasoconstrictive effect of hyperoxia or increased

sympathetic drive, increasing BP [56, 58]. As stated previously, there is a well-defined negative feedback baroreflex mechanism in which BP and HR change in tandem, as demonstrated in the 120s oscillation exposure [59-61]. We also observed increases in RSO_2 which is associated with improvements in CBF. This is likely explained by the possible beneficial influence hyperoxia exerts on cerebral oxygenation and blood flow [67, 69]. However, it must be noted there is no concurrence in the literature about whether or not hyperoxia is "beneficial" for cerebral perfusion, specifically under hypobaric conditions due to a paucity of published data [70, 71]. Lastly, the increase in impedance across time in the 120s oscillation exposure may be indicative of increased pulmonary lymph flow [72]. Indeed, exposure to normoxic hypobaria has been shown to increase intravascular fluid with a compensatory increase in pulmonary lymph [73]. These findings are supported by the non-significant increase in comet tails observed in this cohort. However, it is important to note that any additional pulmonary lymph was not sufficient to compromise Dm.

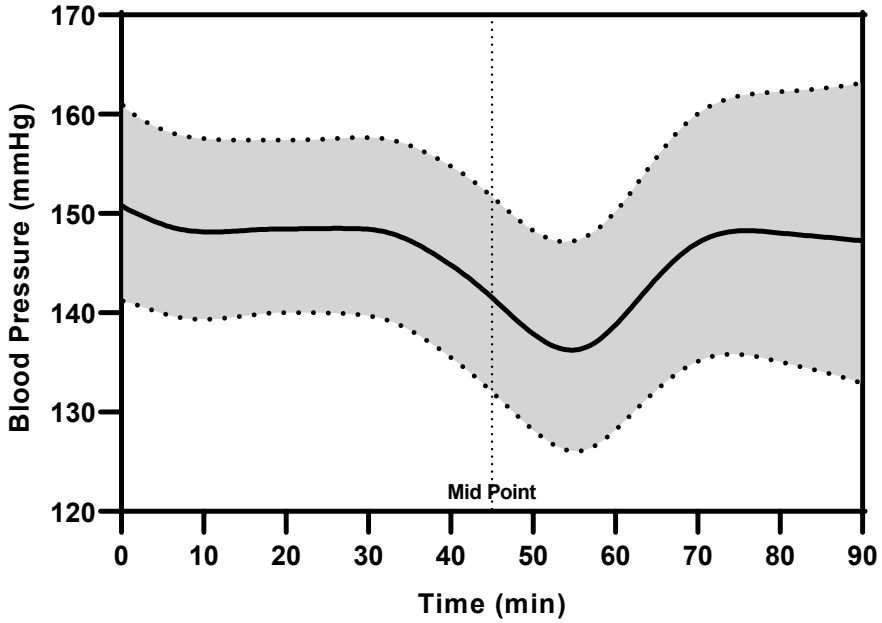


Figure 26. The effect of exposure length on systolic blood pressure during the 120s oscillation rate

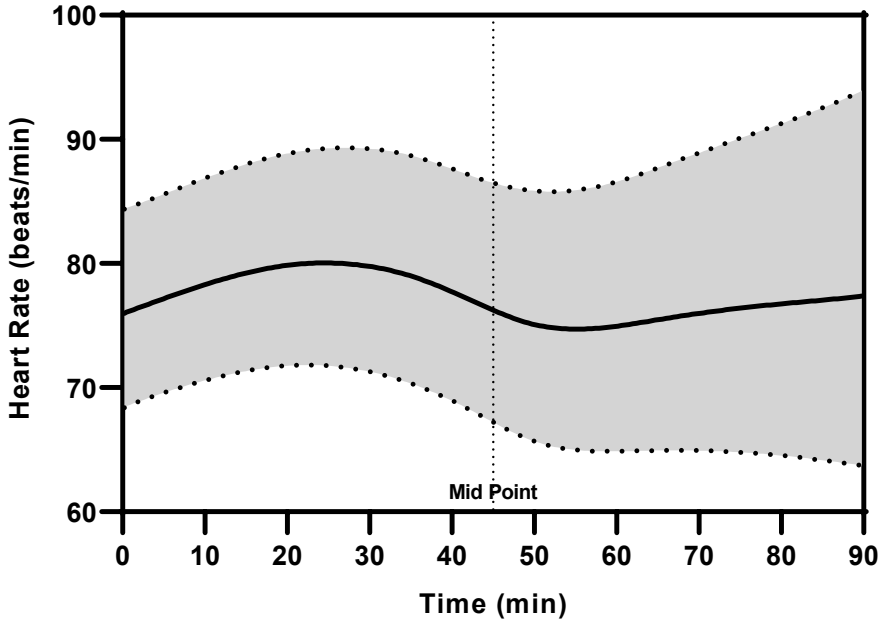


Figure 27. The effect of exposure length on heart rate during the 120s oscillation rate

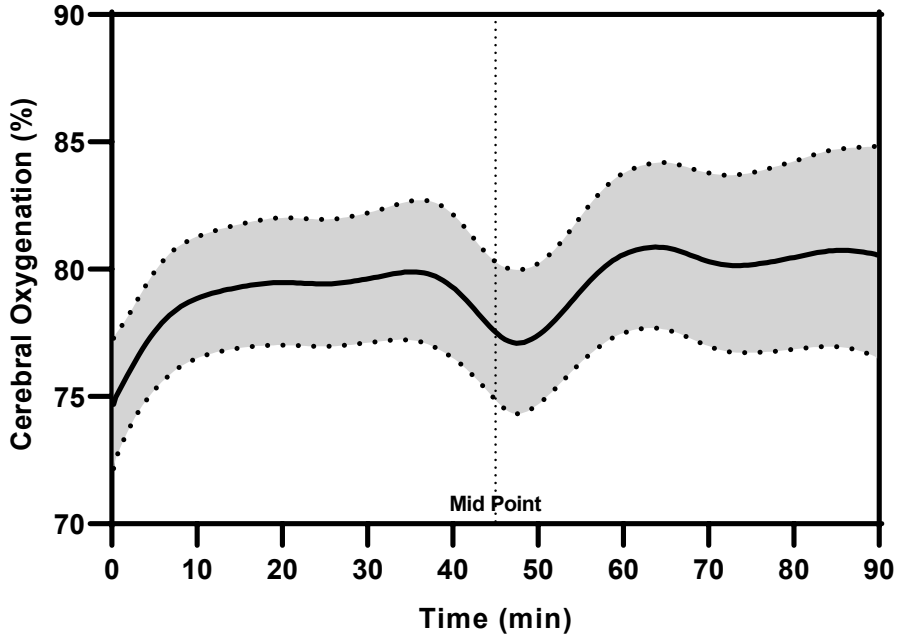


Figure 28. The effect of exposure length on cerebral oxygenation during the 120s oscillation rate

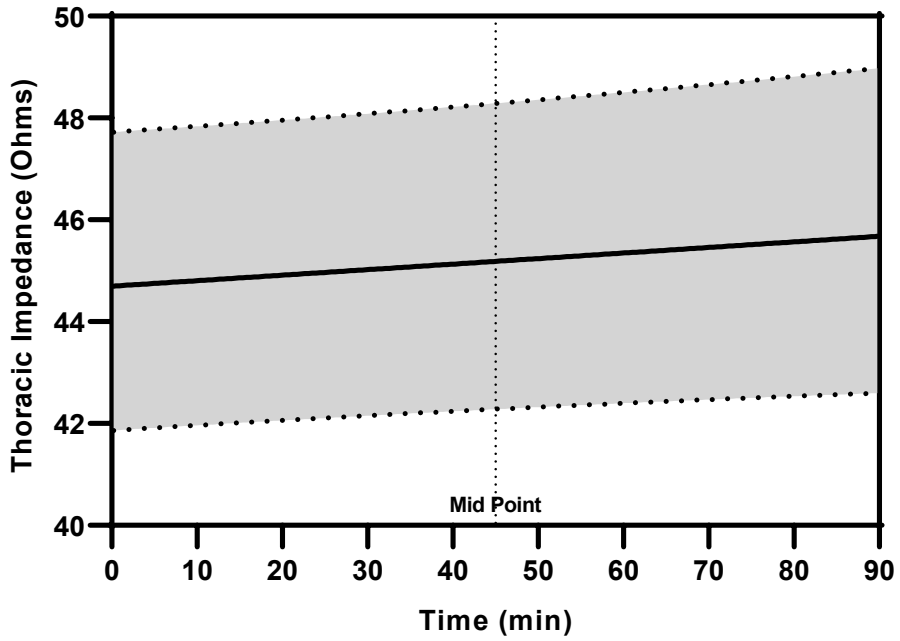


Figure 29. The effect of exposure length on thoracic impedance during the 120s oscillation rate

Table 28. GAMM results for blood pressure during the 120s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.98	0.02	253.60	**<0.001**
s(Time)	7.16	-	35.45	**<0.001**
s(Heart Rate)	0.38	-	0.41	**<0.01**
s(Impedance)	<0.01	-	0.00	**<0.05**
s(Cerebral Oxygenation)	0.69	-	0.53	**<0.001**
s(PETCO2)	<0.01	-	0.00	0.17
s(Cerebral Blood Flow)	1.18	-	83.37	**<0.001**
Random Effects				
ID	4.86	-	2070.49	**<0.001**
ID:Time	3.77	-	805.92	**<0.001**
ID:Heart Rate	<0.01	-	0.00	0.27
ID:Impedance	<0.01	-	0.00	**<0.01**
ID:Cerebral Oxygenation	<0.01	-	0.00	**<0.05**
ID:PETCO2	<0.01	-	0.00	0.17
ID:Cerebral Blood Flow	<0.01	-	0.00	0.29

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on blood pressure (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 29. GAMM results for heart rate during the 120s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.32	0.03	168.60	**<0.001**
s(Time)	4.00	-	9.94	**<0.001**
s(Blood Pressure)	<0.01	-	0.00	0.05
s(Impedance)	1.82	-	1106.29	**<0.001**
s(Cerebral Oxygenation)	<0.01	-	0.00	0.22
s(PETCO2)	0.84	-	4.10	**<0.001**
s(Cerebral Blood Flow)	0.32	-	0.22	**<0.01**
Random Effects				
ID	<0.01	-	0.00	**<0.01**
ID:Time	3.89	-	52.75	**<0.001**
ID:Blood Pressure	3.01	-	244.95	**<0.001**
ID:Impedance	1.70	-	126.97	**<0.001**
ID:Cerebral Oxygenation	<0.01	-	0.00	**<0.01**
ID:PETCO2	<0.01	-	<0.01	**<0.01**
ID:Cerebral Blood Flow	0.30	-	2.99	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; blood pressure was measure in mmHg; impedance was measured in Ohms; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on heart rate (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 30. GAMM results for cerebral oxygenation during the 120s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	4.37	0.01	601.80	**<0.001**
s(Time)	9.64	-	80.00	**<0.001**
s(Heart Rate)	0.11	-	0.63	**<0.05**
s(Blood Pressure)	<0.01	-	0.00	**<0.05**
s(Impedance)	<0.01	-	0.00	0.24
s(PETCO2)	0.88	-	277.46	**<0.001**
s(Cerebral Blood Flow)	<0.01	-	0.00	**<0.01**
Random Effects				
ID	<0.01	-	0.00	0.06
ID:Time	3.94	-	935.17	**<0.001**
ID:Heart Rate	<0.01	-	0.00	**<0.05**
ID:Blood Pressure	<0.01	-	0.00	0.17
ID:Impedance	<0.01	-	0.00	**<0.05**
ID:PETCO2	<0.01	-	0.00	0.05
ID:Cerebral Blood Flow	5.12	-	7609.94	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; impedance was measured in Ohms; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on cerebral oxygenation (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Table 31. GAMM results for thoracic impedance during the 120s oscillation rates.

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	3.81	0.03	129.10	**<0.001**
s(Time)	0.76	-	146.14	**<0.001**
s(Heart Rate)	<0.01	-	0.00	0.61
s(Blood Pressure)	0.89	-	68.30	**<0.001**
s(Cerebral Oxygenation)	2.29	-	226.27	**<0.001**
s(PETCO2)	1.02	-	2.02	**<0.001**
s(Cerebral Blood Flow)	<0.01	-	0.00	
Random Effects				
ID	4.99	-	70548.34	**<0.001**
ID:Time	3.93	-	90.73	**<0.01**
ID:Heart Rate	<0.01	-	0.00	**<0.05**
ID:Blood Pressure	<0.01	-	<0.01	**<0.001**
ID:Impedance	<0.01	-	0.00	**<0.05**
ID:Cerebral Oxygenation	<0.01	-	0.00	0.30
ID:Cerebral Blood Flow	<0.01	-	0.00	0.19

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; heart rate was measured in beats/min; blood pressure was measure in mmHg; cerebral oxygenation was measured in mmHg; PETCO2 was measured in mmHg; cerebral blood flow was measured in mL/min; Bolded *p-values* denote a significant influence of the covariate term on thoracic impedance (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

4.5 Cognitive Testing

There were no differences in RCAT maximum score and accuracy, MCCPT median RT and accuracy, and WLL accuracy in the 30s and 120s oscillation exposures (**Figure 30; Figure 32**). There were no differences in RCAT maximum score and accuracy or WLL accuracy during the 60s oscillation (**Figure 31**). However, we did observe a progressive improvement in WLL mean RTs across all three oscillation exposures. Specifically, WLL mean RTs were significantly delayed in testing period 1 when compared to the other testing times during the 30s oscillations (**Figure 30**). In response to the 60s oscillations, WLL mean RTs were slower for testing period 1 compared

with testing periods 5-10 (**Figure 31**). During the 120s oscillations, WLL mean RTs were significantly longer for testing period 1 and 2 when compared with testing period 3-10 (**Figure 32**). We believe this may be due more to a learning effect than the result of physiologic changes engendered by the exposures. Interestingly, the 60s oscillation exposure was the only one to exhibit significant changes in MCCPT median RT and accuracy. Indeed, MCCPT median RTs were delayed during testing period 8 and 10 compared with testing period 1 and MCCPT accuracy was improved during testing periods 5 and 10 compared with testing period 1 (**Figure 27**). Given the complexity of the WLL test and the apparent stabilization of the mean RTs, we reason this change is more likely a learning effect than a physiological or cerebral alteration in response to the exposures.

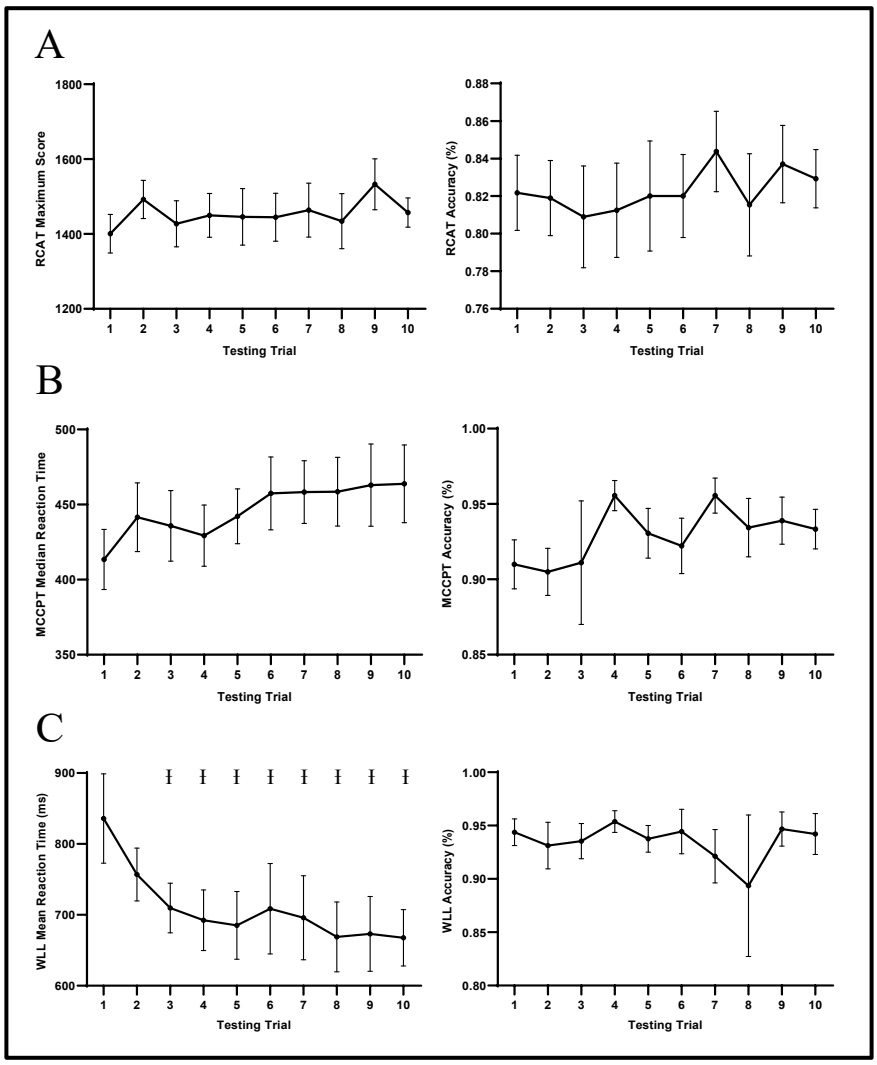


Figure 30. The influence of 30s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on cognitive function. Values represent means ± SD. **Panel 1** denotes RCAT maximum scores and error rates; **Panel 2** denotes MCCPT median hit RTs and error rates; **Panel 3** denotes WLL mean RTs and error rates. Ɨ Significant difference from testing trial 1, $P < 0.05$.

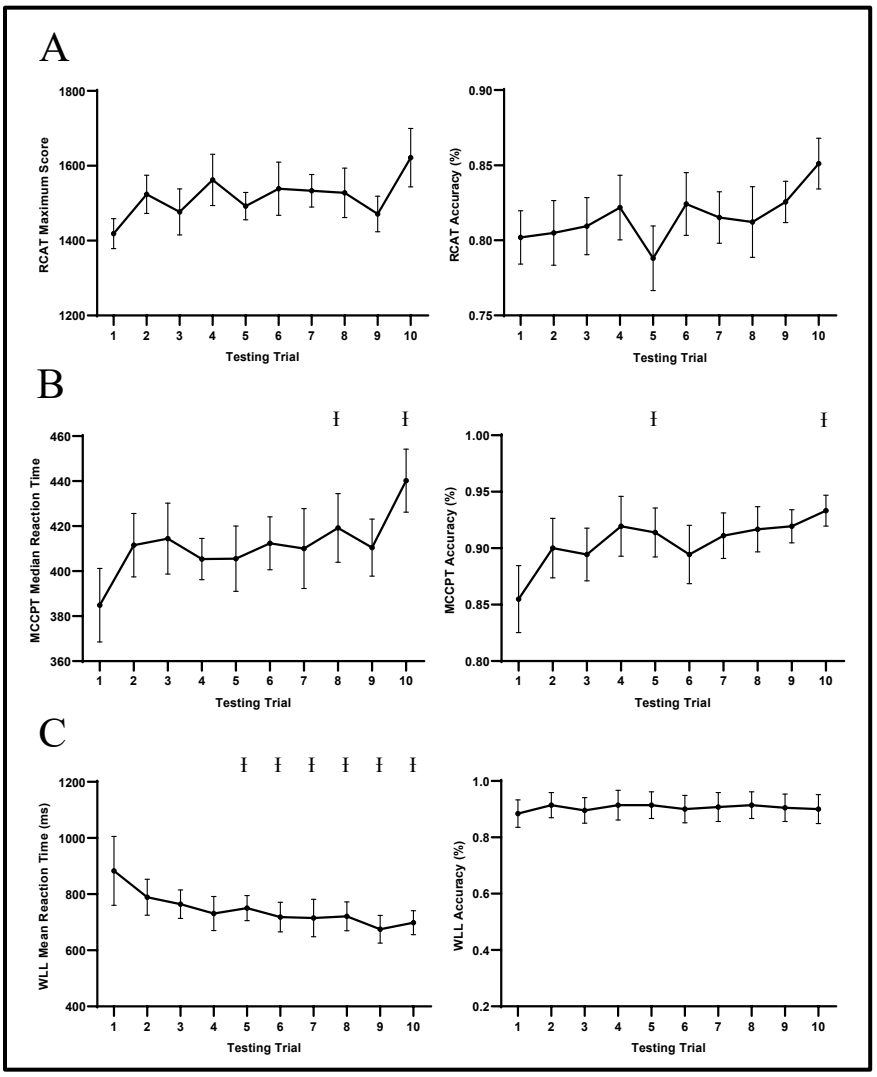


Figure 31. The influence of 60s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on cognitive function. Values represent means ± SD. **Panel 1** denotes RCAT maximum scores and error rates; **Panel 2** denotes MCCPT median hit RTs and error rates; **Panel 3** denotes WLL mean RTs and error rates. Ɨ Significant difference from testing trial 1, $P < 0.05$.

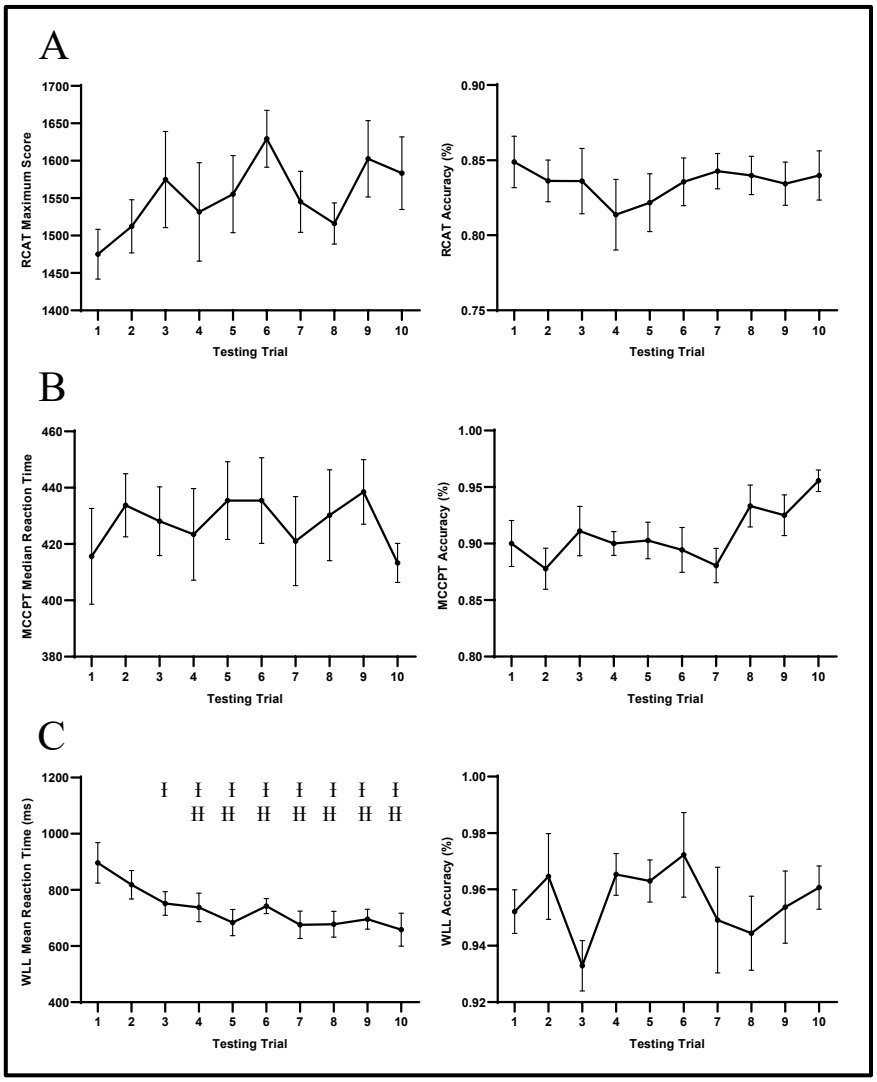


Figure 32. The influence of 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on cognitive function. Values represent means ± SD. **Panel 1** denotes RCAT maximum scores and error rates; **Panel 2** denotes MCCPT median hit RTs and error rates; **Panel 3** denotes WLL mean RTs and error rates. Ɨ Significant difference from testing trial 1, $P < 0.05$. Ħ Significant difference from testing trial 2, $P < 0.05$.

4.6 Implications of Findings

The most robust findings of the present work were that "operationally-relevant" environmental challenges appear to: 1) cause acute, albeit mild decrements in lung physiology with a primary impact on lung mechanics, small changes in the pulmonary vasculature, but with minimal impact on respiratory gas exchange or ventilatory control; and 2) have only minimal influences on cerebral blood flow and oxygenation without apparent negative influences on measures of cognitive performance. These data suggest possible alterations to the peripheral and central chemoreceptor axis in response to oscillating O_2/N_2 concentrations and evidence of alveolar atelectasis and small airway impairment engendered by high inspired O_2. Furthermore, we observed evidence of mild pulmonary-capillary vasoconstriction. While the observed effects of the exposures were small, they may still pose an impediment to optimal pilot performance. Interestingly, the shorter oscillation rates (e.g., 30s) appeared to alter respiratory mechanics to a greater degree whereas longer oscillation rates (e.g., 120s) seem to present more adverse effects on pulmonary function. This may be due to a more rapid alveolar recruitment-derecruitment cycle during shorter oscillations rates or to the shorter time breathing normoxic air, allowing insufficient time for alveolar re-nitrogenation. However, the changes in cardiorespiratory function were quick to resolve after the exposure ended. Interestingly, while we did observe an increase in cerebral oxygenation in response to oscillating O_2/N_2 concentrations, there did not appear to be an effect on cognitive performance. Taken together, the current data demonstrates minimizing inspired gas concentration oscillation rates and amplitude during flight may prove useful in reducing deleterious cardiorespiratory events.

4.7 Further Consideration

Moving forward, we propose the following five suggestions to further assess the influence of oscillating inspires O_2/N_2 concentrations in the background of altitude in an operationally relevant setting: 1) apply chest strapping to impose an elastic load more operationally similar to a flight suit and upper-pressure garment worn by pilots; 2) remove the mid-point testing period to reduce any potential alveolar atelectasis reversal; 3) increase the testing altitude to place further hypobaric stress on the subject; 4) time the ABG draws to occur on the descent of the duty cycle from hyperoxia (i.e., time ABGs to when the subject is actively inhaling high O_2 concentrations) 5) have subjects perform submaximal exercise to reduce the available degrees of freedom when responding to a stressor; and 6) apply high-G acceleration modeling in the background of O_2/N_2 oscillations to determine the benefits of the G suit under a high-G environment in relation to its restrictions placed upon pulmonary function at rest. To the second point, the present data demonstrated several outcome measures returning to baseline after the mid-point testing period. Thus, we believe some of the impact the exposure may have had was reversed during this period of normobaric, normoxic testing. Additionally, any engendered alveolar atelectasis is easily reversed with large breathing maneuvers (e.g., sighing). By removing the mid-point testing period and applying an elastic load by way of chest strapping, it is conceivable that we would cause more atelectasis, and maintain said atelectasis for longer, allowing for easier detection. To the fifth point, applying an external physical demand in the form of exercise, subjects would likely have fewer degrees of freedom to respond to the demands of testing. In response, we believe subjects may not be able to effectively alter respiratory mechanics to compensate for the stressors placed upon them, thus engendering more significant decrements in respiratory physiology. To the last point, while a G suit may present deleterious influences on pulmonary function at rest, it plays an integral role in maintaining

hemodynamics during high-G maneuvers. As such, its benefits to pilot performance may outweigh any detrimental effects on respiratory function. However, to date, there is little research investigating the influence between the benefits of a G suit on hemodynamic responses to high-Gs and the negative influences it may have on respiratory function at rest.

5.0 CONCLUSIONS

While we observed no significant changes in subjects' symptoms ratings, there were more participants within the 120s oscillation group that rated as having *any* symptoms in response to the exposure. Furthermore, there was no evidence of airway inflammation as measured by F_ENO. Despite this, we did observed alterations in respiratory mechanics in response to oscillating O_2/N_2 concentrations. There was evidence decreased peripheral airway compliance in the shorter oscillations, but not the longer oscillations (e.g., 60s and 120s). We propose two possible mechanisms for these findings: 1) this may be a consequence of the more rapid alveolar recruitment-derecruitment cycle associated with the 30s oscillations and why a similar change in Xrs was not seen in the longer oscillatory rates; and 2) this may also be due to the shorter time breathing normoxic air, thus affecting the ability of the lungs to return to baseline PAO_2. Another interesting finding was the reduced operational lung volumes and flow rates observed. Previous literature has demonstrated reduced operational lung volumes which may be indicative of alveolar atelectasis [40, 41]. Additionally, reduced lung volumes are associated with decreased airway reactance [74, 75]. Furthermore, the reduction in flow rates may also point to small airway impairment. In fact, reduced expiratory flow rates have been demonstrated to be highly correlated to small airway disease [42]. Taken together, these data suggest exposure to oscillating O_2/N_2 concentrations may induce alveolar atelectasis and small airway impairment in this cohort. It is important to note that spirometric measures can be highly volitional and, thus, may be influenced

by participant fatigue. However, the researchers adhered to ATS standards for conducting pulmonary function testing and provided ample encouragement to the subjects. As such, we are confident the spirometric measures obtained were accurate.

Our data demonstrated a systemic decline in Q and an associated reduction in Vc. The maintenance of Dm and concomitant reduction in Vc resulted in an increased Dm/Vc. This rise in Dm/Vc may indicate shrinkage of the alveolar-capillary membrane, which may be the result of dehydration. This may be of particular importance as fighter pilots are especially prone to dehydration, particularly in 5th generation fighter aircraft, which can involve sorties of up to 10 hours. These findings suggest exposure high inspired O_2 may result in pulmonary capillary vasoconstriction with little influence on membrane diffusion. Furthermore, there was an observed a systematic reduction in LCI across all exposure types with a return towards baseline at the recovery testing period. In consideration of the reduced Vc, a reduction in LCI may be indicative of alveolar atelectasis. Indeed, a drop off in alveolar units (i.e., complete collapse and/or obstruction) in series may "improve" LCI. This could be due to a lower operational lung volume and less air trapping via fewer lung units capable of ventilation, allowing the inert gas (N_2 in this case) to be cleared with less expired gas. Taken together, these data suggest exposure to oscillating O_2/N_2 concentrations may engender alveolar atelectasis with no attendant influence on the lungs ability to diffuse gases to the blood.

The results of the non-linear modelling suggest an effect of oscillating O_2/N_2 concentrations on peripheral and central chemoreceptor and sympathetic nervous activity. Specifically, we observed an increase in HR within each study arm for all exposure types. Coupled with the decrease in $P_{ET}CO_2$ (30s oscillations) and/or clinical hypocapnia (60s and 120s oscillations) we reason this may be due to the increased sympathetic activity associated with hypocapnia [55]. Additionally,

previous literature has demonstrated an increase in peripheral chemoreceptor activity with resultant downstream sympathetic drive [56]. Interestingly, hypocapnia has been shown to independently depress the central chemoreceptors, which may induce a secondary reflex reduction of the arterial baroreceptors to further drive HR [57]. Further, the data demonstrated an increase in BP during the 30s and 120s oscillations, but no change in the 60s oscillation cohort. This may be partially explained by the direct vasoconstrictive effect of hyperoxia or the increased sympathetic drive associated with acute altitude exposure, increasing BP [56, 58], or that hyperoxia may deactivate the carotid body chemoreceptors [58, 64, 65]. These data suggest a delicate balance between central and peripheral chemoreceptor and sympathetic activity that may be influenced by the length of hyperoxia and/or hypocapnia exposure in the background of mild altitude. Taken together, these data suggest a dynamic relationship between altitude exposure and inspired O_2 concentrations and cardiovascular parameters through modulation of the peripheral central chemoreceptors. Specifically, the hypocapnia induced by exposure to the 30s oscillations may inhibit the central chemoreceptors, resulting in downstream decrease in BP and a compensatory increase in HR. Furthermore, the length of hyperoxic exposure may modulate this relationship. Thus, explaining the increase in HR and the maintenance of BP in response to an increased exposure to hyperoxia (60s vs 30s oscillations). Interestingly, as hyperoxic exposure length increases, it appears baroreflex activity may be preferentially increased as demonstrated by the emergence of a negative feedback baroreflex mechanism in which BP and HR change in tandem in the 120s oscillation exposure [59-61].

Our data also demonstrated alterations to RSO_2., CBF, and thoracic impedance in response to exposures to oscillating O_2/N_2 concentrations. We observed mild decreases in CBF with concomitant increases in RSO_2. These findings are in agreement with previous literature wherein

exposure to hyperoxia has been shown to increase cerebral oxygenation with minimal impact on CBF [67, 69, 70]. Interestingly, hypocapnia (a consistent feature in our cohorts) has been demonstrated to decrease cerebral oxygenation and blood flow [66]. As such, it appears exposure to hyperoxia exerted a stronger influence on cerebral oxygenation and CBF than did hypocapnia in the present study. Lastly, we observed an increase in impedance across time in the 120s oscillation exposure. This increase in thoracic impedance may be indicative of increased pulmonary lymph flow [72]. Indeed, exposure to normoxic hypobaria has been shown to induce increased intravascular fluid with a compensatory increase in pulmonary lymph [73]. This increase in pulmonary lymph flow is further supported by the small increases in comet tails observed in our cohort (5.9 vs 7.0, 6.4 vs 7.6, and 7.1 vs 9.6 for 30s, 60s, and 120s oscillations respectively).

Taken together, the observed changes in lung mechanics suggest mild alveolar atelectasis and/or mild changes in airway tone; the reduction in pulmonary capillary blood volume suggest derecruitment due to a small fall in Q or altered vascular tone (reduced NO production or mild hypocapnia); and the slight changes in ventilatory control may be evidence of an altered balance between peripheral and central chemoreceptors. The findings of this work suggest exposures to exposures to shorter oscillations in O_2/N_2 concentrations may present the most adverse effects on respiratory mechanics whereas longer oscillations appear to have more adverse effects on pulmonary function while at mild altitude, but in context, the overall influence or challenge to the respiratory system appears relatively mild. We propose two primary mechanism for these findings. First, the relatively short exposures to normoxia in all oscillation frequencies may not have been sufficient time to recover N_2 gas tensions, not allowing to a return to baseline PO_2. As such. short oscillation rates may in fact expose participants to longer times with elevated PO_2 which may have a compounding influence wherein consistently elevated PO_2 may induce more decrements to

respiratory function. Second, we reason the combination of hyperoxia and altitude exposure may alter the relationship between the peripheral and central chemoreceptors and downstream sympathetic drive. As such, these relatively short oscillation exposures may further increase sympathetic drive, causing more negative cardiovascular impacts.

The current data demonstrates minimizing inspired gas concentration oscillation rates and amplitude during flight may prove useful in reducing deleterious cardiorespiratory events in high-performance aircraft pilots. Given these results, consideration should be given to instructing pilots to perform long, controlled breaths to prevent hypocapnia and occasional deep breaths to reverse the formation of any atelectasis. Furthermore, we would suggest the implementation of pre- and post-sortie testing to evaluate cardiorespiratory function and determine if there were any decrements to cardiorespiratory function as a result of said sortie. This may provide more information as to the emergent alterations in cardiorespiratory function engendered by the operational environment and guide protocols for the amount of “down time” prescribed to each pilot to ensure optimal recovery and performance.

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APPENDIX A. Supplementary materials

Supplementary Table 1. The influence of 30s, 60s, and 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on fractional exhaled nitric oxide.

Testing Period	Mean	SD	*p-value*
30s Oscillation Rate			
Pre	13.00	5.31	
Mid	11.67	3.50	0.57
Post	12.56	4.13	0.92
Recovery	12.33	4.53	0.85
60s Oscillation Rate			
Pre	14.70	13.16	
Mid	12.70	10.59	0.47
Post	11.10	10.13	0.06
Recovery	11.2	8.15	0.07
120s Oscillation Rate			
Pre	17.00	7.84	
Mid	11.67	6.80	0.08
Post	13.00	6.78	0.26
Recovery	15.22	11.09	0.84

SD: standard deviation. Means are report in parts per billion. There was no difference in fractional exhaled nitric oxide at any testing period.

Supplementary Table 2. The influence of 60s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on airway resistance and reactance at 5, 11, and 19Hz.

Testing Period	Mean	SD	*p-value*
		5Hz Resistance	
Pre	2.69	0.82	
Mid	2.86	0.72	0.86
Post	2.89	0.71	0.79
Recovery	2.81	1.06	0.94
		5Hz Reactance	
Pre	–0.62	0.26	
Mid	–0.55	0.13	0.83
Post	–0.63	0.24	0.99
Recovery	–0.66	0.29	0.97
		11Hz Resistance	
Pre	2.69	0.88	
Mid	2.81	0.63	0.93
Post	2.89	0.73	0.73
Recovery	2.73	0.94	0.99
		11Hz Reactance	
Pre	0.20	0.19	
Mid	0.20	0.14	0.99
Post	0.17	0.20	0.95
Recovery	0.15	0.20	0.88
		19Hz Resistance	
Pre	2.68	0.84	
Mid	2.82	0.62	0.88
Post	2.89	0.70	0.66
Recovery	2.73	0.88	0.99
		19Hz Reactance	
Pre	0.97	0.34	
Mid	0.95	0.23	0.99
Post	0.88	0.29	0.79
Recovery	0.87	0.26	0.73

SD: standard deviation. Resistance and reactance are reported in Ohms. There were no differences in airway resistance or reactance at any frequency.

Supplementary Table 3. The influence of 120s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on airway resistance and reactance at 5, 11, and 19Hz.

Testing Period	Mean	SD	*p-value*
		5Hz Resistance	
Pre	2.89	1.18	
Mid	2.61	1.05	0.34
Post	2.97	0.91	0.97
Recovery	2.79	0.88	0.93
		5Hz Reactance	
Pre	–0.52	0.20	
Mid	–0.40	0.28	0.14
Post	–0.50	0.18	0.99
Recovery	–0.54	0.24	0.98
		11Hz Resistance	
Pre	2.95	1.22	
Mid	2.61	1.07	0.19
Post	2.97	0.91	0.99
Recovery	2.77	0.87	0.72
		11Hz Reactance	
Pre	0.16	0.19	
Mid	0.19	0.23	0.92
Post	0.15	0.25	0.99
Recovery	0.11	0.27	0.85
		19Hz Resistance	
Pre	2.97	1.13	
Mid	2.61	0.97	0.18
Post	2.98	0.79	1.00
Recovery	2.79	0.84	0.70
		19Hz Reactance	
Pre	0.84	0.26	
Mid	0.85	0.24	0.99
Post	0.81	0.36	0.97
Recovery	0.74	0.34	0.65

SD: standard deviation. Resistance and reactance are reported in Ohms. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

Supplementary Table 4. The influence of 60s oscillations of 80/20 and 30/70 O_2/N_2 concentrations on lung diffusion measures.

Testing Period	Mean	SD	*p-value*
Membrane Diffusion Capacity (mL/min/mmHg)			
Pre	45.12	7.52	
Mid	47.07	8.32	0.65
Post	46.49	10.65	0.84
Recovery	48.09	8.05	0.30
Pulmonary-Capillary Blood Volume (mL)			
Pre	110.85	22.23	
Mid	101.30	18.50	0.11
Post	100.20	17.68	0.06
Recovery	104.08	21.17	0.36
Dm/Vc (1/min/mmHg)			
Pre	0.43	0.09	
Mid	0.48	0.08	0.28
Post	0.48	0.07	0.45
Recovery	0.48	0.07	0.27
Cardiac Output (mL/min)			
Pre	5.39	1.08	
Mid	5.09	1.07	0.69
Post	5.45	1.29	0.99
Recovery	5.29	1.32	0.98

SD: standard deviation. Bolded *p-values* denote a significant difference between that testing period and the pre exposure testing period (P <0.05).

LIST OF SYMBOLS, ABBREVIATIONS, AND ACRONYMS

Akaike's information criteria	AIC
American thoracic society	ATS
Alveolar – capillary conductance	Dm
Alveolar-arterial difference	A-a difference
Alveolar partial pressure of oxygen	PAO_2
Airway reactance	Xrs
Airway resistance	Rrs
Arterial blood gases	ABG
Arterial oxygen tension	PaO_2
Cardiac output	Q
Dead space volume	V_{ds}
Dead space volume of the rebreathe bag	$V_{ds,rb}$
Diffusing capacity for carbon monoxide	DLCO
Diffusing capacity for nitric oxide	DLNO
Expiratory reserve volume	ERV
Forced expiratory volume in 1 second	FEV1
Fractional exhaled NO	F_ENO
Forced oscillation of the airways	FOT
Forced vital capacity	FVC
Functional residual capacity	FRC
Generalized additive mixed effects models	GAMM
Height	Ht
Hematocrit	Hct
Hemoglobin	Hb
Lung clearance index	LCI
Masked conjunctive continuous performance task	MCCPT
Maximal forced expiratory flow	FEF_{max}
Minute ventilation	VE
Near-infrared spectroscopy	NIRS
Partial pressure of end-tidal CO_2	$P_{ET}CO_2$
Partial pressure of oxygen	PO_2
Pulmonary blood flow	Vc
Pulmonary function testing	PFT
Rapid cognitive assessment task	RCAT
Ratio of physiologic dead space over tidal volume	VD/VT
Reaction time	RT
Reactive oxygen species	ROS
Repeated measures analyses of variance	ANOVA
Respiratory equivalent	RQ
Respiratory rate	RR
Restricted maximal likelihood	REML
Cerebral oxygenation	RSO_2
Slow vital capacity	SVC
Total lung capacity	TLC
Total systemic volume	$V_{s,tot}$
Tidal Volume	Vt
Transcranial doppler	TCD

Unexplained physiological events	UPE
Ventilator-induced lung injury	VILI
Volume of the rebreathe bag	V_{rb}
Weight	Wt
Well living lab	WLL

TASK 2: THE COGNITIVE COST OF INCREASED WORK OF BREATHING (PHASE II)

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1.0 SUMMARY

There are growing concerns over the unexpected physiological events among pilots of multiple US Air Force and US Navy high-performance aircraft. In April 2012, the Restrictive Breathing Working Group (RBWG) identified that aircraft life support systems may impose an excessively high work of breathing (Wb) on pilots during flight operations. Furthermore, the Navy Experimental Diving Unit (NEDU) recently suggested that reducing the Wb may protect against physiological incidents for fighter pilots in the future. Additionally, our most recent DoD/USAF-funded project entitled "The Cognitive Performance Cost of Increased Work of Breathing" (Task Order: 0052; Grant number: FA865012D6280) suggests reaction time was positively associated with inspiratory effort sensation during inspiratory loading trials, and that errors were negatively associated (i.e., improved) with increasing expiratory effort sensation during expiratory resistive loading. These findings support our rationale that a heightened *inspiratory* Wb experienced by jet pilots may constitute a "distraction stimulus" consequent to an increased sensation of respiratory muscle effort. However, there are multiple forms of respiratory loading that are placed on a pilot during flight. In addition to resistive loading, one such type of loading is threshold loading wherein the pilot must produce a certain level of respiratory pressure to produce flow. As such, the purpose of this study was to determine the extent to which increasing respiratory muscle effort (via threshold loading breathing) impacts cognitive performance, specifically in the domain of attentional focus.

Twelve, healthy participants (Age: 29 ± 6 yr) were recruited for this study. Participants visited the laboratory on 3 separate occasions. *Visit 1* consisted of routine pulmonary function testing, and familiarization with the Masked Conjunctive Continuous Performance Task (MCCPT) – the psychometric tool we used to strain central processing and assess sustained and selective

attentional performance. During *Visit 2*, participants completed 6 repetitions of a shortened MCCPT protocol while breathing against 4 different inspiratory threshold loads (i.e., no, light, moderate, and heavy loads). During *Visit 3*, participants completed a similar series of MCCPT trials, excepting that 4 different threshold loads were added during expiration. Throughout the experiments, participants breathed on a 2-way nonrebreathing valve to separate the inspiratory and expiratory circuits. Inspiratory and expiratory flows were measured using separate heated pneumotachographs. A humidifier was arranged in-series with the inspiratory limb of the circuit. A computer-controlled variable resistor was used to set the inspiratory or expiratory threshold loads.

Repeated measures analysis of variances (ANOVAs) were used to demonstrate: (i) the efficacy of the threshold loading device to impose significantly higher loading at each loading condition; (ii) the effects of loading condition on respiratory muscle effort sensation; and (iii) the influence of hypercapnia on MCCPT scores during inspiratory and expiratory threshold loading. Inspiratory threshold loading significantly augmented ($P < 0.05$) inspiratory effort sensation and pressure-time product (PTP). Expiratory threshold loading significantly increased expiratory effort sensation, PTP, and end-tidal O_2, and increased end-tidal CO_2 ($P < 0.05$). The MCCPT response accuracy decreased as the expiratory threshold load increased ($P < 0.05$). There was also a tendency for reaction time to increase at the highest of the imposed expiratory loads. These findings indicate that the threshold loading device was effective at imposing a load sufficient to augment PTP, end-tidal CO_2, and respiratory muscle effort.

Generalized additive mixed effects models (GAMMs) were used to examine the effects of respiratory muscular effort sensation, device loading, and hypercapnia, on the nonlinear trends in MCCPT scores during inspiratory and expiratory threshold loading. These analyses revealed that

median hit reaction time (RT) was positively associated with inspiratory effort sensation during inspiratory loading trials. A similar relationship was demonstrated for expiratory effort sensation and median hit RT during expiratory loading trials. Furthermore, median hit RT was negatively associated with end-tidal CO_2 during expiratory loading trials.

The findings of this work suggest that it was not increasing respiratory muscle effort (i.e., PTP) that impacts attentional performance, but rather, the subjects' perception of the respiratory load. Indeed, both inspiratory and expiratory effort sensation, independent of PTP, significantly impacted central processing independent of PTP ($P < 0.001$). Simply, as effort sensation increases, so too did median hit RT. Furthermore, there was a strong, negative association between end-tidal CO_2 and median hit RT in the expiratory loading condition, but not during the inspiratory loading conditions ($P = 0.29$ and $P < 0.05$, for inspiratory and expiratory loading conditions respectively). These current findings suggest relative hypoventilation may be deleterious to RTs while relative hyperventilation may confer a selective benefit to RTs, but only during expiratory loading. These most recent findings are in accordance with our previous findings detailed in the DoD/USAF-funded project entitled "The Cognitive Performance Cost of Increased Work of Breathing" (Task Order: 0052; Grant number: FA865012D6280). As such, it is reasonable that minimizing respiratory effort sensation (independent of the mechanical output of the respiratory muscles) during flight operations may prove useful in reducing pilot RTs during complex behavioral tasks.

2.0 INTRODUCTION

Higher performance aircraft are a vital component in US homeland defense and are a major contributor to the Nation's air dominance and superiority during joint military (defensive and offensive) operations. These high-performance aircraft are capable of imposing supra-physiologic perturbations on pilots owing to their super cruise capabilities, super-manoeuvrability, stealth, and embedded/integrated avionics system. Despite the superlative features of these high-performance aircraft, it has become apparent over the past decade that they are not designed for pilot optimization such that flying these aircraft have posed a number of serious concerns for pilot health and safety. Indeed, there occurred a rising number of physiological incidents among high-performance aircraft pilots between the years of 2008 to 2011, the upward trend of which ultimately lead to a fleet wide stand-down in May 2011 [1]. Additionally, there exists a lack of understanding of the physiology associated with the new extremes in flight envelope. In response to the growing concern over pilot safety, several investigations and task forces were assembled to identify ways in which to optimize pilot performance and, where possible, eliminate sources of great risk to pilots. In due course, several findings and recommendations were offered, namely those pointing out system-specific factors in the onboard oxygen delivery system. However, in April 2012, the Restrictive Breathing Working Group (RBWG) highlighted a previously unaddressed problem affecting pilot safety: namely that the F-22 Life Support System imposed an excessively high mechanical work of breathing [1]. In fact, as a result of an investigation led by the Navy Experimental Diving Unit (NEDU), it was suggested that reducing Wb may protect against physiological incidents for high performance aircraft pilots during flight operations in the future.

As stated above, the RBWG indicated that the F-22 Life Support System provides an "excessively"

high Wb to the pilot during flight operations. It must not be forgotten, however, that working in the operational aerospace environment, *per se*, imposes unique demands on the respiratory system of the high-performance aircraft pilot. For example, the extreme accelerative forces exerted on the thorax, such as that encountered during tactical high-G maneuvers, decrease chest wall compliance [2, 3] and increase the elastic Wb [for review see ref #4]. Exposure to high-G loads may also lead to "acceleration atelectasis" [5, 6] which, in turn, may reduce regional lung compliance and further increase the elastic Wb. Moreover, the anti-G straining maneuvers used to protect against loss of consciousness may, in and of themselves, impose a significant mechanical load on the respiratory muscles over time [7]. Taken together, it is evident that breathing is an energetically demanding task for the high-performance aircraft pilot during flight operations. Yet, while it may be clear that a high Wb is implicated in the manifestation of high-performance aircraft pilot physiological issues, the precise *mechanisms* by which an elevated Wb impacts on pilot safety remain elusive. We argue below that the perception of increased respiratory muscle effort (consequent to a high Wb) may directly impair cognitive performance of the high-performance aircraft pilot.

The act of breathing is largely an unconscious experience: rarely do we perceive the muscular effort required to breathe while at rest. However, under circumstances where the mechanical load imposed on the respiratory muscles is elevated, the sensation of breathing effort may increase to the point where it can no longer be ignored [8]. Such elevated Wb may be perceived as "increased breathing effort/discomfort", "air-hunger", "unsatisfied inspiration" or "chest tightness" [8, 9]. Stated in other words, high levels of respiratory muscle work impinge on the consciousness of the individual, and is often experienced as a negative (noxious or pain-related) affective sensation [8-10]. Because elevated respiratory muscle effort may occupy a *nontrivial* portion of the conscious experience, it follows that a requisite amount of cognitive resources must be devoted to "paying

attention" to this noxious stimuli [11]. Indeed, our most recent DoD/USAF-funded project entitled "The Cognitive Performance Cost of Increased Work of Breathing" (Task Order: 0052; Grant number: FA865012D6280) suggest increasing inspiratory effort sensation may prolong the period of central information processing during complex reaction time tasks. We argue that an increased perception of respiratory muscle effort may directly impair cognitive performance of the jet fighter pilot. The findings of this study were that central processing speed (reaction time) progressively lengthened (worsened) the greater that inspiratory muscle "effort" sensation increased across the loading trials. Moreover, there was a tendency for error-rate to increase (decrease in response accuracy) at the highest of the expiratory resistive loads (~100 $cmH_2O/L/s$). Based on these findings, we suggested that attempts to minimize inspiratory effort sensation (independent of device resistance) during flight operations may prove useful in reducing pilot reaction times during complex behavioral tasks, especially when only tight margins of error can be tolerated.

It remains to be determined whether other "operationally-relevant" respiratory loads may cause similar decrements in cognitive performance. Other "pilot-specific" respiratory loads may include those caused by inflation of the upper-body pressure garment (UPG) during High-G aerial maneuvers (chest wall restriction), and/or the additional respiratory pressures that a pilot must develop to generate airflow through the pilot's mask and/or the Onboard Oxygen Generation System (pressure-threshold loads). With specific reference to the latter, the primary objective of this work was to evaluate the impact of increasing the Wb on cognitive performance in healthy adults. The Wb was augmented by the addition of threshold loads during either inspiration or expiration, separately. The magnitude of the threshold load was adjustable via a computer-controlled adjustable poppet valve. Cognitive performance was assessed within the domain of attentional performance via a modified masked conjunctive continuous performance task

(MCCPT). It was hypothesized that under circumstances of increased threshold loading, the augmented perception of respiratory muscle effort would compete for available cognitive resources, impairing subjects' attentional performance on the MCCPT. Given that there is no clear understanding of the relationship between the Wb and cognitive performance, the outcomes of this research will address a key gap in current knowledge, for which there is high relevance to pilot safety and performance. More directly, the findings of this research may be used to guide modifications/updates to air breathing standards for high-performance aircrafts.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

3.1 Participants

Twelve healthy male participants (Age: 29 ± 6 yr) were recruited for this study. Participants had no known history of cardiac, pulmonary, and/or metabolic disease, and no reported mental or psychological disorders of attention. Each participant completed both the inspiratory and expiratory loaded breathing visits except for 1 participant who did not return for their expiratory visit (n = 12 and n = 11, for inspiratory and expiratory loaded breathing visits respectively). Additionally, one participant was removed from the analysis due to technical difficulties during data collection (n = 12 and n = 10 for inspiratory and expiratory loaded breathing visits, respectively). The present study conformed to the principles outlined in the Declaration of Helsinki and was approved by the Mayo Clinic Internal Review Board.

3.2 Experimental Design

To determine the impact of increasing the inspiratory or expiratory threshold respiratory effort on attentional performance, participants visited the laboratory on 3 separate occasions. A description

of the methods and procedures for each visit is provided below.

3.2.1 Visit 1 – Familiarization. During this visit, pulmonary function testing was performed and participants were familiarized with the Masked Conjunctive Continuous Performance Task (MCCPT) – the psychometric tool we used to strain central processing and assess sustained and selective attentional performance [12]. In brief, the MCCPT is a complex choice reaction time (RT) task that requires participants to either respond or withhold a response to a visual stimulus. The visual stimulus consisted of a colored mask, comprised of four superimposed figures (circle, square, triangle, and hexagon) in different colors (red, blue, yellow, and green). To avoid habituation effects, minor movements (e.g., "jittering" of the image) in which every 10–20 ms two mask-images were alternated, one of which had thicker borders around the superimposed figures. The mask appeared at the center of the screen and disappeared when it was replaced by either a target or distractor shape for 100 ms. The mask then reappeared immediately, generating a pre- and post-masking of each target and distractor. The target shape was a red circle and distractor stimuli were either similar in shape (blue and yellow circle), similar in color (red hexagon and red triangle), or completely different (blue hexagon and yellow triangle). All target and distractor shapes appeared at the center of the screen with an inter-stimulus interval between 2,000 and 5,000 ms. The task was to provide a response via a key press on a mechanical switch as fast as possible for all shapes and colors that were not a red circle. Subjects were instructed to withhold from providing a response when presented with a red circle. We developed a novel microcontroller-based device to implement the MCCPT – the device provided RT values with a sub-millisecond accuracy.

The original version of the MCCPT, as developed by Shalev et al. [12], takes approximately 20 min to complete. However, it was not feasible to apply a given inspiratory/expiratory threshold

load for this length of time to our participants. This point is particularly important given that it was our intention to examine attentional performance across various, and potentially heavy loads. Thus, we modified the original long version of the MCCPT by dividing the protocol into six 2½ minute sequences (~40 stimulus presentations in each trial). In this manner, we could apply a given threshold load for a relatively brief duration of time, and, through the 6 repetitions, we were able to accumulate the necessary number of stimulus responses to compute the RT and error rate scores as per the original long version of the MCCPT.

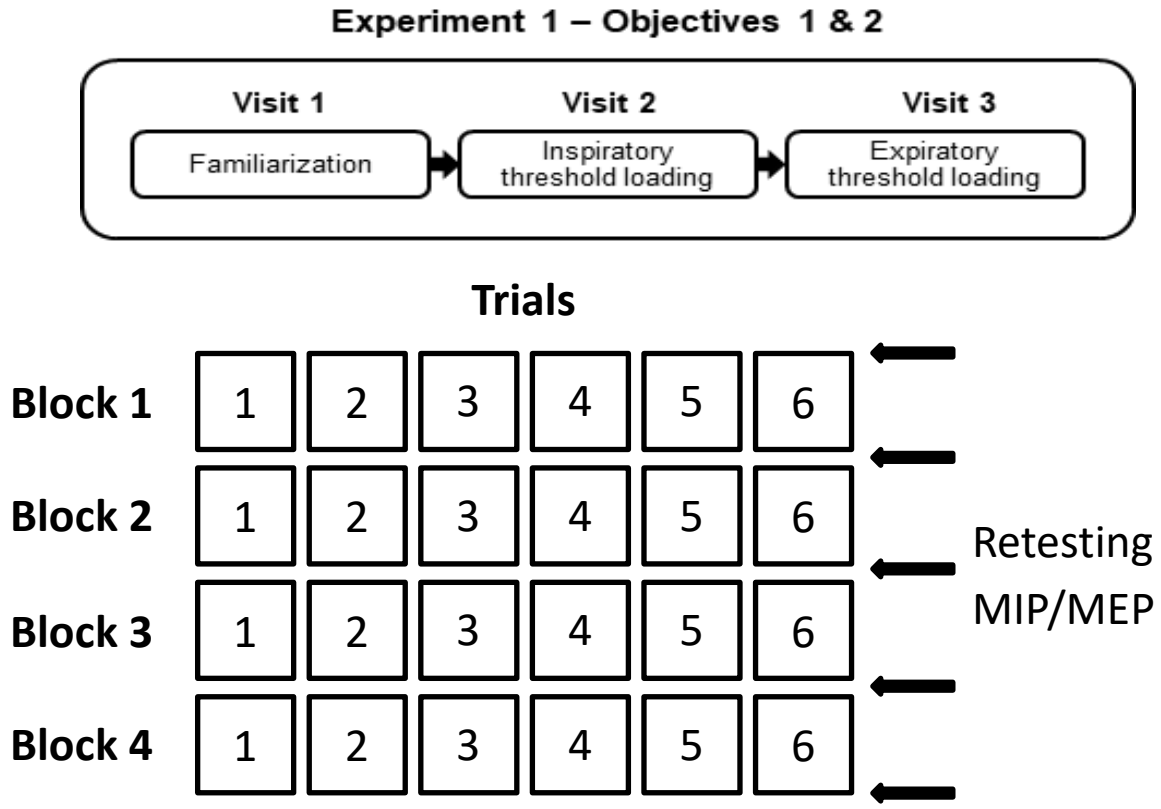
3.2.2 Visit 2 – Inspiratory Threshold Loaded Breathing. The study flow for Visit 2 is presented in **Figure 1**. Participants breathed on a 2-way nonrebreathing valve to separate the inspiratory and expiratory circuits. Inspiratory and expiratory flows were measured separately using heated pneumotachographs (3813 series, Hans Rudolph, KS, USA). A humidifier was arranged in-series with the inspiratory limb of the circuit. A computer-controlled adjustable poppet valve was interposed between the inspiratory port of the 2-way nonrebreathing valve and the humidifier. This valve was adjustable via a custom-built software interface and was used to set the threshold load during each trial. The participant was instructed to complete 24 trials of the MCCPT protocol (40 visual stimuli per trial). During each trial of this MCCPT protocol, one of four loads were added to the inspiratory circuit in such a way that the peak inspiratory mouth pressure achieved either <5%, ~10%, ~20%, or ~40% of the recorded baseline maximal inspiratory pressures (MIPs), notated here as loads 1 (control), 2, 3, and 4. These loads were imposed surreptitiously in randomized order such that 6 repetitions of each load were presented across the course of the visit (i.e., 24 total trials). Immediately after each trial was completed, the participant was asked to rate their perceived inspiratory muscle effort required to breathe against the imposed load on the modified 10-point category ratio scale (CR10; [13]). Approximately 2 min of rest was given

between each trial. Participants wore noise-cancelling headphones to reduce the effects of ambient noise and environmental distraction on their MCCPT performance. Every 6 trials, the participant was given a longer break (~10 min) where they were taken off the mouthpiece and were free to move and walk around. During each 6-trial run, however, participants were instructed to remain on the mouthpiece. Maximal inspiratory pressures were obtained at regular intervals during the visit to assess whether inspiratory muscle fatigue was evident.

Due to the nature of the MCCPT, we could not provide any visual or auditory feedback on breathing pattern and respiratory muscle effort for fear of distracting the participant from the task at hand. As such, participants were free to adopt any rate or depth of breathing they felt most comfortable during each loading condition (1-4). The 4 loads were determined before experimental data collection began, on a participant-by-participant basis. The investigator varied the load imposed by the computer-controlled variable threshold device until the peak mouth pressure swing was approximately 10% of the recorded baseline MIP – this condition was set as load 2. Loads 3 and 4 were determined in similar fashion by adjusting the variable resistor until peak inspiratory mouth pressure swings were ~20% and ~40% of baseline MIP, respectively. Load 1 was set at minimal load with the poppet valve opened to its maximal aperture (i.e., control condition). It is emphasized that the determination of each load (1 through 4) was performed while the participant was practicing the MCCPT protocol. Because participants were spontaneously breathing during the loaded trials (i.e., no feedback was given), the peak mouth pressure swings for a given "intended" load were liable to change slightly over the course of the 24 experimental trials. Hence, it was sometimes necessary to adjust the resistance at a given load to bring the peak inspiratory mouth pressure swing back into the desired range. Although a rare occurrence, if any adjustments were necessary, they were performed *between* and not *during* trials.

3.2.3 Visit 3 – Expiratory Threshold Loaded Breathing. The procedures of Visit 3 were identical to Visit 2, excepting that the threshold loads were instead added to the expiratory circuit of the breathing apparatus, and that maximal expiratory pressures (MEPs) were obtained at regular intervals throughout the visit. The "intended" expiratory loads were determined in similar fashion to that described above for Visit 2, excepting that Loads 2, 3 and 4 were adjusted until peak expiratory mouth pressure were approximately 10%, 20%, and 40% of the recorded baseline MEP, respectively.

Figure 1. Study design



3.3 Measured and Computed Variables

3.3.1 Respiratory Pressures and Mechanics. Mouth pressure was sampled via a lateral port located in the mouthpiece. Inspiratory and expiratory flows were measured separately using heated pneumotachographs (3813 series, Hans Rudolph, KS, USA). A humidifier was arranged in-series with the inspiratory limb of the circuit such that the inspired air had an approximate humidity of 100%. Respiratory muscle effort was expressed as the pressure-time product (PTP) which was quantified as the product of the average inspiratory mouth pressure (Pi_{avg}) and the duration of

inspiration (T_i) or the average expiratory mouth pressure (Pe_{avg}) and the duration of expiration (T_i) for inspiratory and expiratory phases respectively.

For inspiratory PTP:

$$PTP = Pi_{avg} \times Ti \quad (1)$$

For expiratory PTP:

$$PTP = Pe_{avg} \times Te \quad (2)$$

A positive value for PTP denotes inspiratory muscle pressure-development, whereas a negative PTP represents expiratory muscle pressure-development.

3.3.2 End-tidal Gases, Pulse Oxygenation and Heart Rate. The partial pressures of O_2 and CO_2 ($P_{ET}O2$ and $P_{ET}CO2$, respectively) were measured via a rapid-response O_2/CO_2 analyzer (GA-200B, iWorx, NH, USA) from a sample line placed in the expiratory limb of the experimental breathing circuit. Pulse oxygenation was measured via the fingertip of the non-dominant hand (Radical 7, Massimo, CA, USA). Heart rate and rhythm was recorded using a single-channel bio-amplifier module (FE132, ADInstruments, NSW, AUS).

3.3.3. Respiratory effort sensation. The Borg 10-point category ratio scale (CR10) was used to assess subjective ratings of respiratory effort sensation during each loaded breathing trial. An example of the scale used in these experiments is provided in **Table 1**. The scale was explained to the participant in detail, using standard protocol [13]. Participants were asked to specifically rate their feelings of "effort" required to breathe against the device resistance, and to ignore other qualitative perceptions such as "air hunger", chest tightness, fear of suffocation, the sensation of muscular "tension" required to breathe, and any other uncomfortable sensation that they may perceive during the trials (i.e., stiffness due to being seated for a prolonged duration, etc.).

Table 1. The Borg Category Ratio (CR10) Scale to assess Respiratory Effort Sensation

0	Nothing at all	
0.3		
0.5	Extremely weak	Just noticeable
0.7		
1	Very weak	
1.5		
2	Weak	Light
2.5		
3	Moderate	
4		
5	Strong	Heavy
6		
7	Very strong	
8		
9		
10	Extremely strong	"Maximal"
11		
∫		
●	Absolute maximum	Highest possible

3.3.4 Masked Conjunctive Continuous Performance Scores: The principal measurement used in the computation of the continuous performance scores of the MCCPT is participants' reaction time (RT) in response to the stimulus presentation (i.e., target or distractor). The accurate measurement of RT is therefore dependent on the degree of accuracy with which both stimulus presentation and the responding mechanical keypress can be recorded. To this end, our custom-built microcontroller device measured the onset of stimulus presentation via a light sensor attached to the LCD computer display. The mechanical keypress was readily detected as a switching state from high to low on a digital input port of the microcontroller. The time elapsed between these

two events was measured via an interrupt-driven routine that was able to provide elapsed durations with sub millisecond accuracy. The microcontroller device communicated with a host PC via USB, such that trial correctness could be matched with the RT measured by the microcontroller device. Each stimulus presentation was coded into one of the following 2 categories, depending on the shape and color of the stimuli:

Conjunctive = Stimuli with the same shape or color to the target

- Color Conjunctive = Stimuli with the same color as the target
- Shape Conjunctive = Stimuli with the same shape as the target

Non-conjunctive = Stimuli with a different shape and/or color to the target

Only correct responses were used in the calculation of hit RT values. RT values were excluded from analysis if the observed value was <200 ms or ≥1000ms. The resulting distributions of RT values for each participant were typically non-normal and, as such, median RTs for the above conditions were computed for each loading condition, separately. Additional parameters computed were error types (i.e., commission and omission); the ability to discriminate the target from the distractor (d'), which incorporates the two error types – commissions and omissions; and the criteria (β) which provides a measure of the balance between error types.

$$d' = z(\text{hit rate}) - z(\text{false alarm rate}) \quad (3)$$

$$\beta = \text{Covariance}/\text{Variance} \quad (4)$$

Omission errors refer to a subject not responding to a stimulus the subjects *was* supposed to (e.g., not responding to a red triangle) whereas a commission error refers to a subject responding to a

stimulus the subject was *not* supposed to (i.e., responding to a red circle). For β, a positive value means a higher tendency towards omission errors, and vice versa (when β value is zero, there is no bias towards any particular error type).

3.4 Statistical Approach

3.4.1 Effect of Threshold Loading on Masked Conjunctive Continuous Performance Scores.

The measured and computed variables obtained during the 6 repetitions of each load were averaged to provide a single value per load, per subject. Repeated measures analyses of variance (ANOVAs) were used to determine the effect of increasing threshold load (1, 2, 3 and 4) on respiratory mechanics, breathing pattern, end-tidal gases, and MCCPT performance. Data obtained for inspiratory (Visit 2) and expiratory (Visit 3) threshold loading trials were analyzed using separate repeated measures ANOVAs – no effort was made to compare data between these two experimental conditions.

Generalized additive mixed effects models (GAMMs) were used to determine the impact of respiratory mechanics, respiratory effort sensation, and end-tidal partial pressure of CO_2 ($P_{ET}CO_2$) on MCCPT performance scores. In brief, the GAMM model is similar to multiple linear regressions, insofar as it attempts to model the independent (main) effects of inputted covariates on the outcome variable (e.g., Median Hit RT). However, an important assumption of multiple linear regression is that all observations are independent, uncorrelated with each other, and demonstrate linearity – an assumption that is flatly violated with repeated measures data, such as in our study. The GAMM model, on the other hand, handles repeated observations by robustly accounting for the correlation between observations that are clustered within each subject. Additionally, the GAMM allows for the fitting of non-linear smoothing splines to individual,

repeated measures. Thus, the GAMM model allows for non-linear modeling and provides group-level parameter estimates of covariate main-effects after accounting for the within-subject correlation between observations. The parameter of respiratory mechanics that was chosen as a covariate in these GAMM models was the empirically determined inspiratory and expiratory PTP generated in response to the load imposed by the adjustable poppet valve during each loading condition. We opted for this parameter because it can be more readily obtained under operational conditions (i.e., cockpit) compared with those variables that require instrumentation with esophageal and gastric balloon catheters and measurement of esophageal pressure (P_{es}) (e.g., Wb, etc.). Additionally, $P_{ET}CO_2$ and $P_{ET}O_2$ were calculated as a change from baseline to account for the variability in subject resting end-tidal partial pressures. Statistical significance was considered if $P < 0.05$.

The GAMM models used in this study were selected through the interrogation of multiple competing models. Additionally, competing distributional families were compared using the Akaike information criterion (AIC) to determine which family was most appropriate. Through these comparisons, we determined a log linked Gamma distribution most closely fit the data for the reaction time models, and a beta regression family most closely fit the data for the error rate models. Included in these models were main effects and random effects for patient ID, respiratory effort sensation, PTP, $P_{ET}CO_2$, and $P_{ET}O_2$. It is important to note that while hyper- and hypocapnia have been associated with altered cognitive performance, there are conflicting reports as to their relationship (i.e., positive or negative) [14-16]. The presence of altered $P_{ET}CO_2$ and the current uncertainty of how altered arterial CO_2 may affect cognition, were driving forces behind our decision to include $P_{ET}CO_2$ in our GAMM models.

The selection of group-level main effects and interaction terms was determined using a backward selection method based on the Akaike's Information Criteria (AIC) score [17]. The final GAMM model was fit using the restricted maximal likelihood (REML) method, cubic regression penalties for nonlinear smooths, the hyperparameter γ was calculated using BIC-like parameters (i.e., log(n)/2) to reduce overfitting, and a false discover p-value adjustment to reduce false positives [18, 19]. An extra penalty was added to each individual term so it could be penalized to zero, thereby allowing terms to be automatically "selected out" from the GAMM when appropriate.

4.0 RESULTS AND DISCUSSION

4.1 Inspiratory Threshold Loading

4.1.1 Inspiratory Mouth Pressures, Pressure-Time Product, Effort Sensation, and Ventilatory Parameters. The inspiratory pressure-time product, and inspiratory effort sensation, together increased with augmenting inspiratory loads (**Figure 2**; $P < 0.001$). There were no signs of significant inspiratory muscle fatigue throughout Visit 2, as evidenced by the steady values of maximal inspiratory pressure (MIP) following the different loading trials shown in **Figure 2**. The peak and mean inspiratory mouth pressure swings during the inspiratory threshold loading conditions are presented in **Figure 3.** There was a load-dependent rise in the magnitude of peak inspiratory mouth pressure swings ($P < 0.001$). Furthermore, there was a similar pattern of increasing mean inspiratory mouth pressure was observed with augmenting inspiratory threshold loads ($P < 0.001$). These observations are important because they serve to confirm that, on average, we successfully maintained the participant's spontaneous peak inspiratory mouth pressure swings within the "intended" ranges for each loading condition (see Section 3.2.2 for further details). Taken together, these findings confirm that inspiratory muscle pressure-development was

progressively increased in response to the rising threshold loads. Although the imposed threshold loads were indeed large, our participants did not appear at risk of developing inspiratory muscle fatigue – an observation which corroborates the stable MIP values observed across loading conditions (**Figures 2**). We are thus confident that our approach to determining, and imposing the 4 different loads did, in fact, engender separate inspiratory threshold pressures and evoked unique increases in the sensation of inspiratory muscle effort.

Given that participants were free to adopt any rate and depth of breathing during the MCCPT trials, there was a certain degree of variability in the breathing pattern response to inspiratory threshold loading. Nonetheless, there were some observable trends that were mostly evident at the higher levels of inspiratory resistance (e.g., load 2 and 3). Minute ventilation increased with augmenting inspiratory load (**Figure 4**). This increased minute ventilation was the result of increases in tidal volume rather than breathing frequency wherein tidal volume was increased in conditions 2, 3, and 4 when compared to load 1 ($P \leq 0.001$). Interestingly, this relative hyperpnea was not accompanied by significant changes in $P_{ET}CO_2$ nor $P_{ET}O_2$ (**Figure 4)**. Our findings are consistent with the literature, insofar as larger inspiratory threshold loads may alter ventilatory patterns but not to such an extent as to influence end-tidal gases [20, 21].

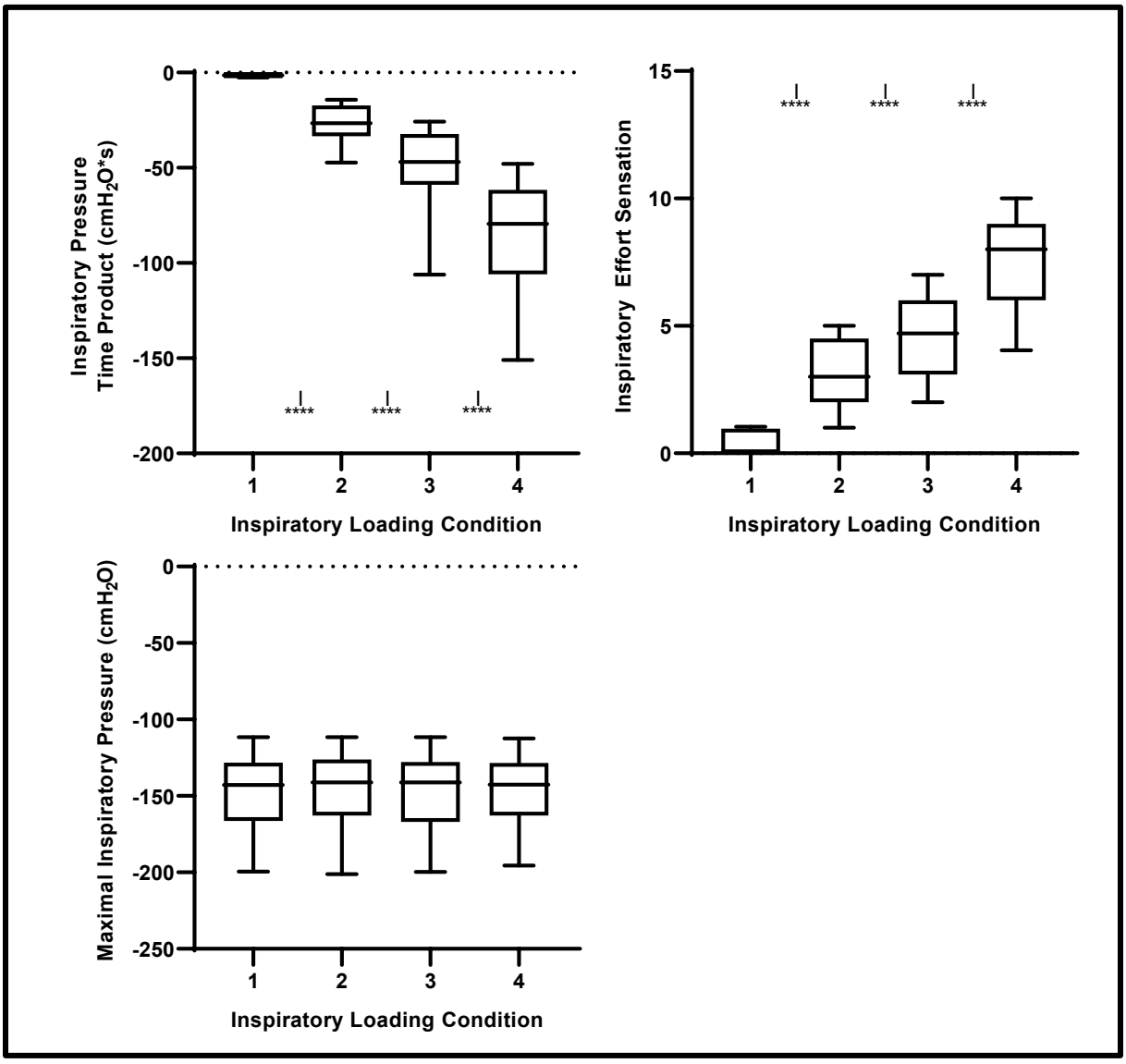


Figure 2. Inspiratory pressure-time product, effort sensation and maximal inspiratory pressure (MIP) across inspiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. **** Significant difference from previous load condition, $P < 0.001$. Ł Significant difference from load condition 1, $P < 0.05$.

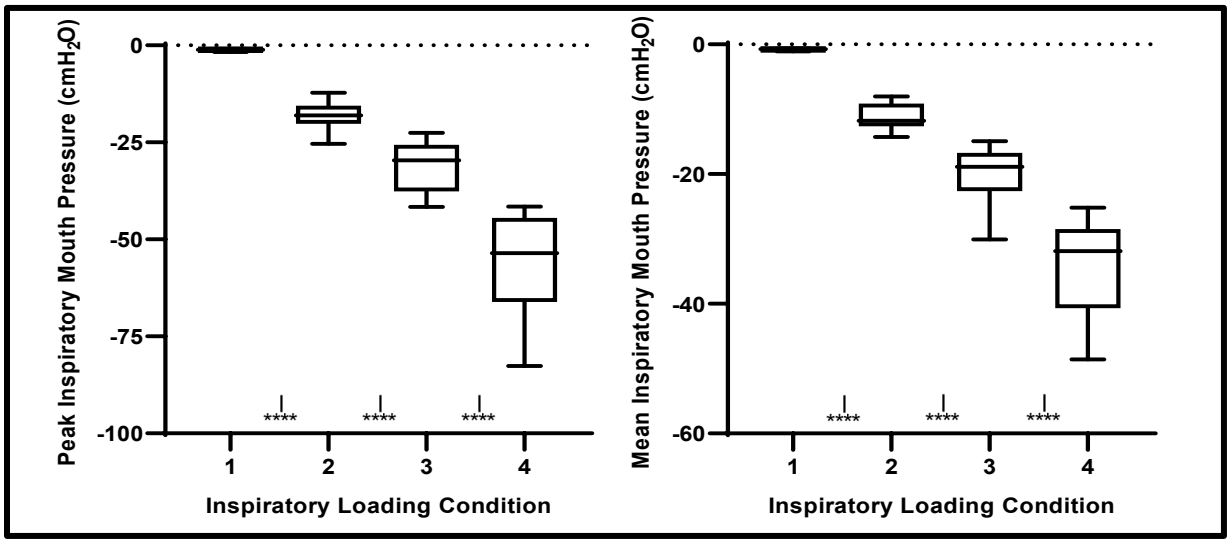


Figure 3. Peak and mean inspiratory mouth pressure swings across inspiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. **** Significant difference from previous load condition, $P < 0.0001$, Ł Significant difference from load condition 1, $P < 0.05$.

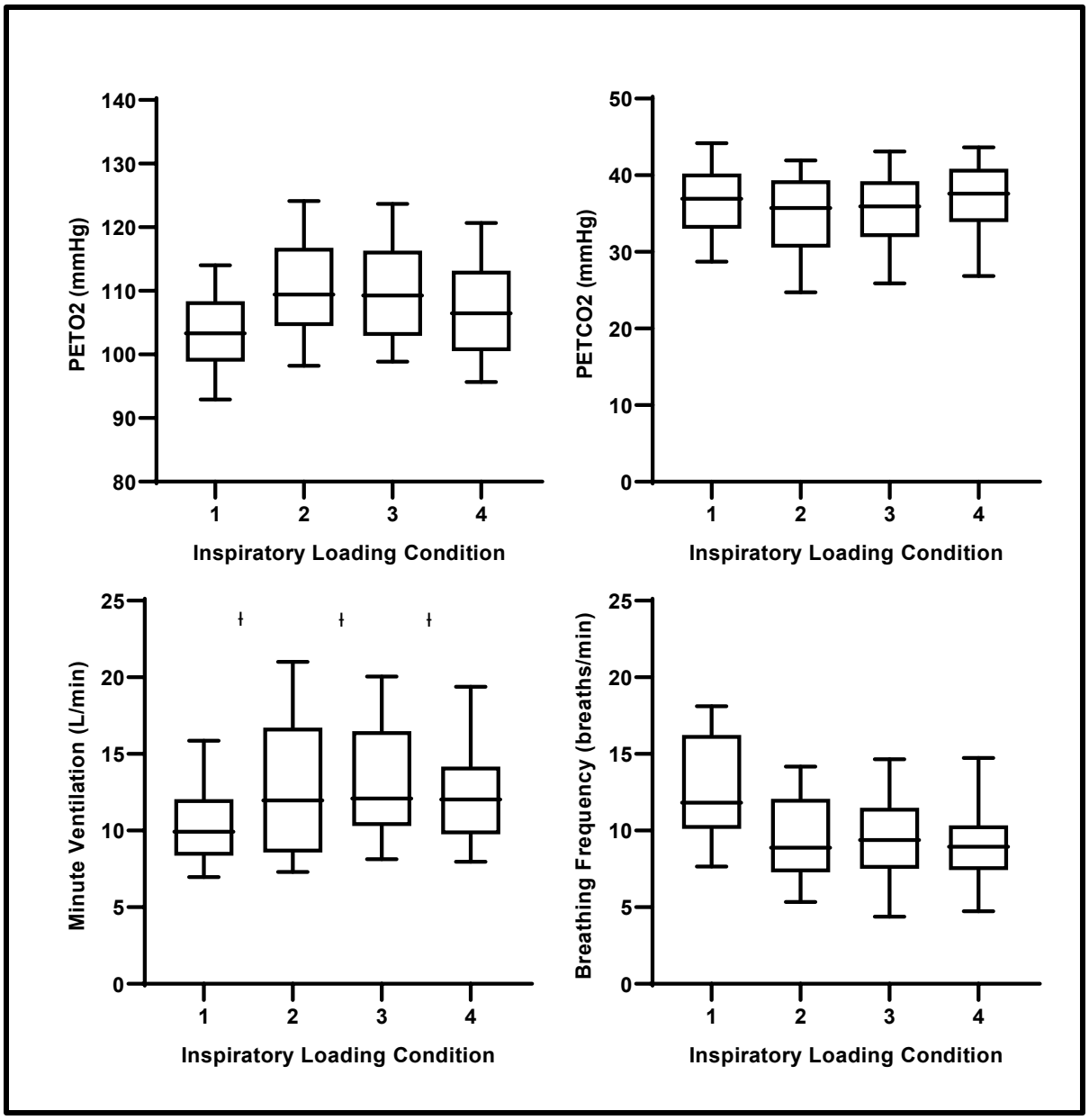


Figure 4. End-tidal gases and ventilatory responses to inspiratory threshold loading during masked conjunctive continuous performance trials. Values represent means ± SEM. $P_{ET}O_2$ and $P_{ET}CO_2$: end-tidal partial pressures of O_2 and CO_2. Ɨ Significant difference from load condition 1, $P < 0.05$.

4.1.2 Masked Conjunctive Continuous Performance Task Results During Inspiratory Loading. There were observable changes in MCCPT performance across inspiratory threshold loading conditions – wherein median hit RT was significantly longer during loads 2, 3, and 4 compared with load 1 (**Table 2, Figure 5**; $P < 0.05$). However, there were no differences in omission nor commission error rates between the inspiratory loading conditions. (**Figure 6**). While there were no differences in the overall error rates, how the stimuli was presented did influence RT – wherein median hit RT during load 4 was significantly higher for color conjunctive ($P = 0.031$), shape conjunctive ($P < 0.001$), and non-conjunctive ($P < 0.01$) distractor stimuli when compared to load 1. Furthermore, median hit RT was higher for the shape conjunctive distractor stimuli when compared to load 2 ($P = 0.012$). These data suggest that at higher inspiratory threshold loads, participants had difficulty identifying the target and discriminating it from the distractors. These findings support our hypothesis, insofar as breathing on high inspiratory loads may stress attentional resources preventing the participants from continuously engaging in the MCCPT, thus diminishing perception and delaying RT [22].

Table 2. Median Hit Reaction Times Across Inspiratory Threshold Loading Conditions.

Loading Conditions			
Condition	**Mean**	**SD**	***p-value***
1 (No Load)	441.4	81.9	
2 (Light Load)	468.3	80.6	**0.049**
3 (Moderate Load)	471.4	83.9	**0.024**
4 (Heavy Load)	481.7	108.6	**0.002**

SD: standard deviation. Bolded *p-values* denote a significant difference between the inspiratory and expiratory load and load 1 ($P < 0.05$).

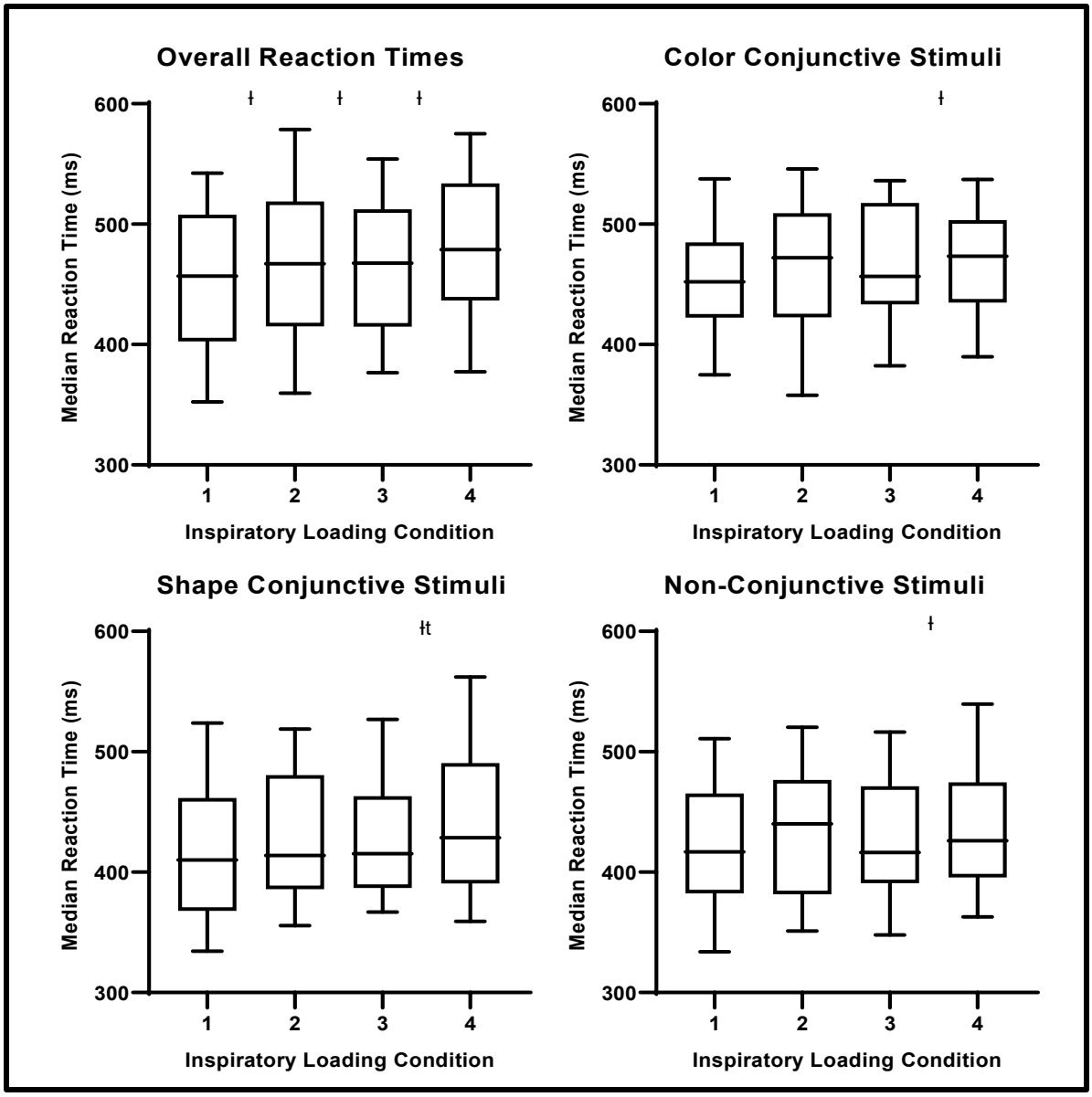


Figure 5. Median reactions times for different stimuli across inspiratory threshold loading conditions during masked conjunctive continuous performance trials. V es represent means ± SEM. Ɨ Significant difference from load condition 1, $P < 0.05$. t Significant difference from load condition 2, $P < 0.05$.

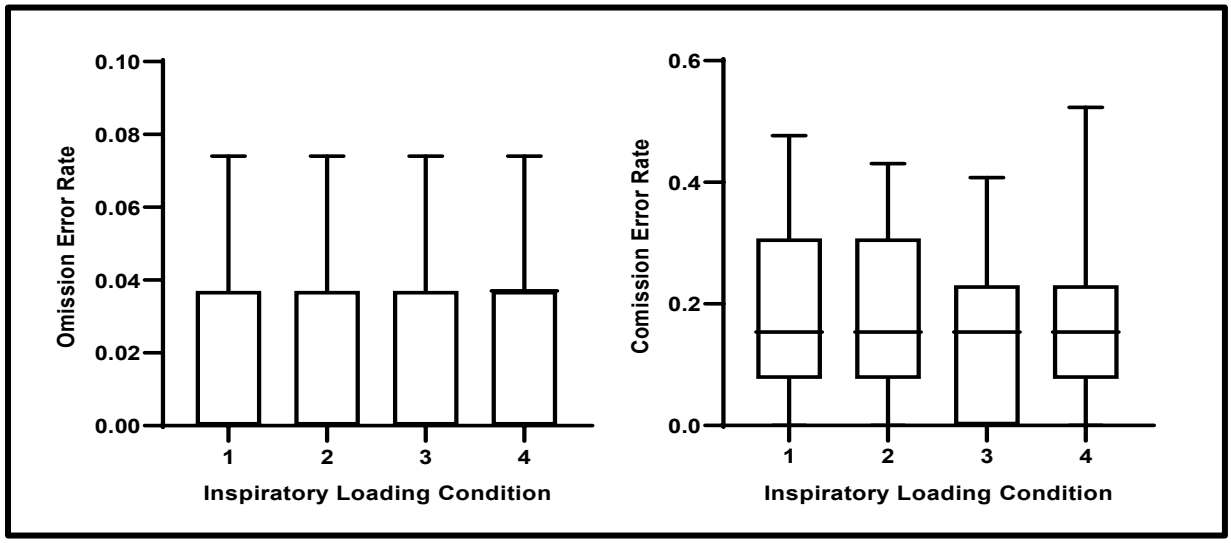


Figure 6. Error rates across inspiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. There were no differences in error rates between loading conditions.

4.2 Expiratory Threshold Loading

4.2.1 Expiratory Mouth Pressures, Pressure-Time Product, Effort Sensation, and Ventilatory Parameters. The expiratory pressure-time product, and expiratory effort sensation, together increased with augmenting expiratory threshold loads (**Figure 7**; P <0.001). There were no signs of significant expiratory muscle fatigue throughout Visit 3, as evidenced by the steady values of maximal expiratory pressure (MEP) in **Figure 7**. The peak and mean expiratory mouth pressure swings during the expiratory threshold loading conditions are presented in **Figure 8.** As with the inspiratory loading trials, there was a load-dependent rise in the magnitude of peak expiratory mouth pressure swings (P < 0.001). Additionally, a pattern of increasing mean expiratory mouth pressure was observed with augmenting expiratory loads (P < 0.001). These observations are important because they demonstrate we were able to successfully maintain the participant's spontaneous peak expiratory mouth pressure swings within the determined ranges for each loading condition (see Section 3.2.2 for further details). Taken together, these findings confirm that expiratory muscle pressure-development was progressively increased in response to the rising threshold loads. Furthermore, our participants did not seem to be at risk of developing expiratory muscle fatigue, which is supported by the stable MEP values observed across loading conditions (**Figures 7**). We are thus confident that our approach to determining and imposing the 4 different loading conditions did, in fact, engender separate expiratory threshold pressures and evoke unique increases in the sensation of expiratory muscle "effort".

As demonstrated in the inspiratory threshold loading trials there was variability in the breathing pattern response to expiratory threshold loading. However, there was a clear influence of expiratory threshold loading on breathing pattern wherein minute ventilation decreased with increasing expiratory threshold load (**Figure 9**). This decrease in minute ventilation was the result

of a decreased breathing frequency rather than tidal volume, wherein breathing frequency during loads 2, 3, and 4 were significantly lower than load 1 ($P < 0.001$). Not surprisingly, these altered respiratory patterns were accompanied by an increased $P_{ET}O_2$ and a fall in $P_{ET}CO_2$ (**Figure 9)**. These findings are consistent with previous literature in that imposing a high expiratory load results in participants adopting a ventilatory pattern consisting of increased end-expiratory lung volumes as well as breathing at elevated lung volumes [23, 24]. Taken together, we posit the participants in this study may have undertaken a breathing pattern characterized by breathing at higher lung volumes in an effort to use chest wall recoil pressures to aid in expiration and "avoid" the expiratory load. These findings are consistent with our previous DoD/USAF-funded project entitled "The Cognitive Performance Cost of Increased Work of Breathing" (Task Order: 0052; Grant number: FA865012D6280) in that participants spontaneously adopted a respiratory pattern consisting of large inhalations in an effort to create high chest wall recoil pressures to overcome the expiratory threshold load. And in doing so, "ride out" the expiration. The authors caution this interpretation of these results as we did not instrument our participants with esophageal and gastric balloon catheters to adequately measure chest wall or plural pressures.

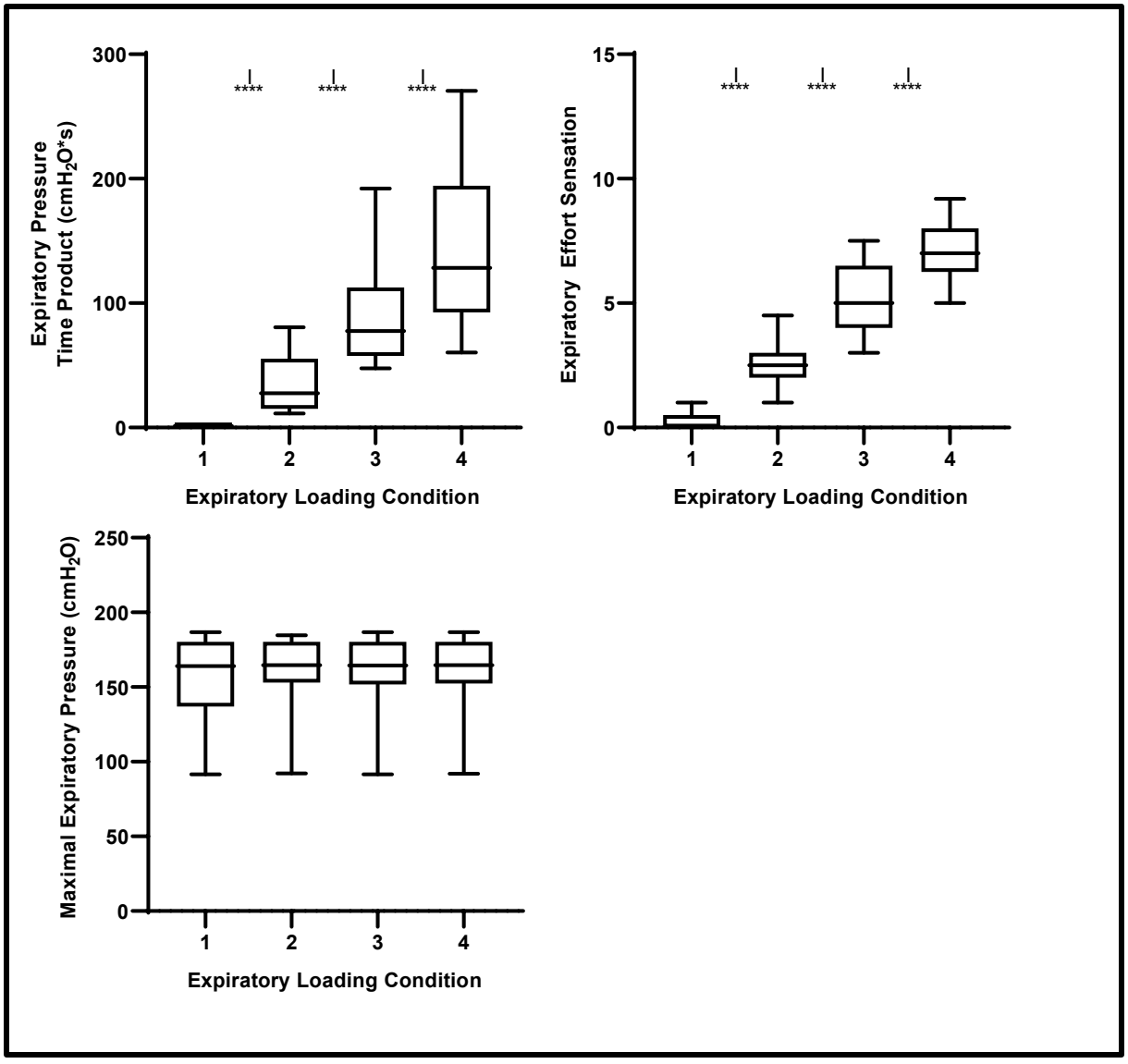


Figure 7. Expiratory pressure-time product, effort sensation and maximal expiratory pressure (MEP) across expiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. **** Significant difference from previous load condition, $P < 0.001$. Ł Significant difference from load condition 1, $P < 0.05$.

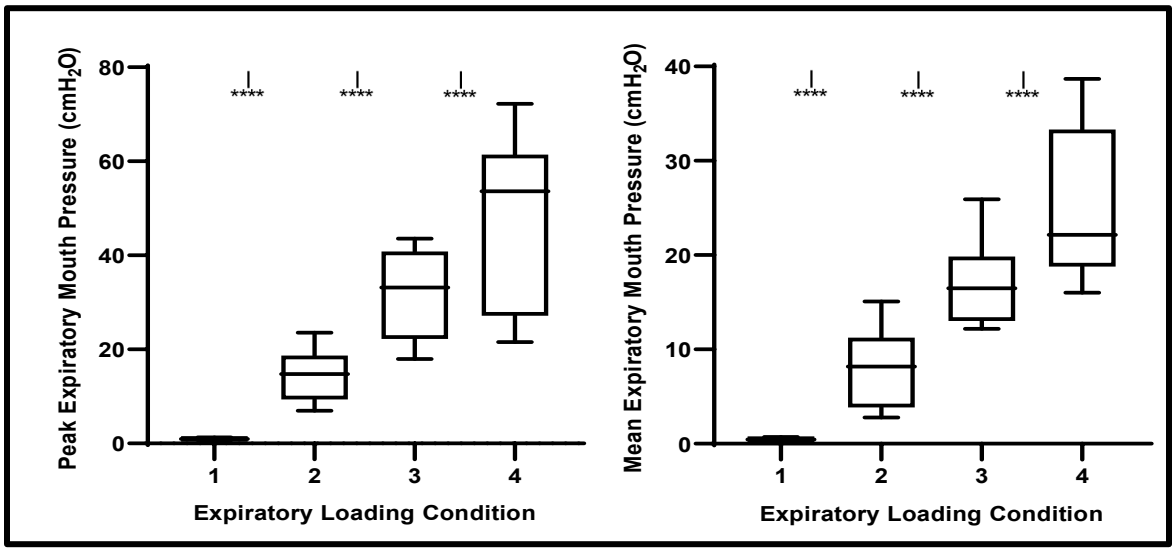


Figure 8. Peak and mean expiratory mouth pressure swings across expiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. **** Significant difference from previous load condition, $P < 0.0001$, Ł Significant difference from load condition 1, $P < 0.05$.

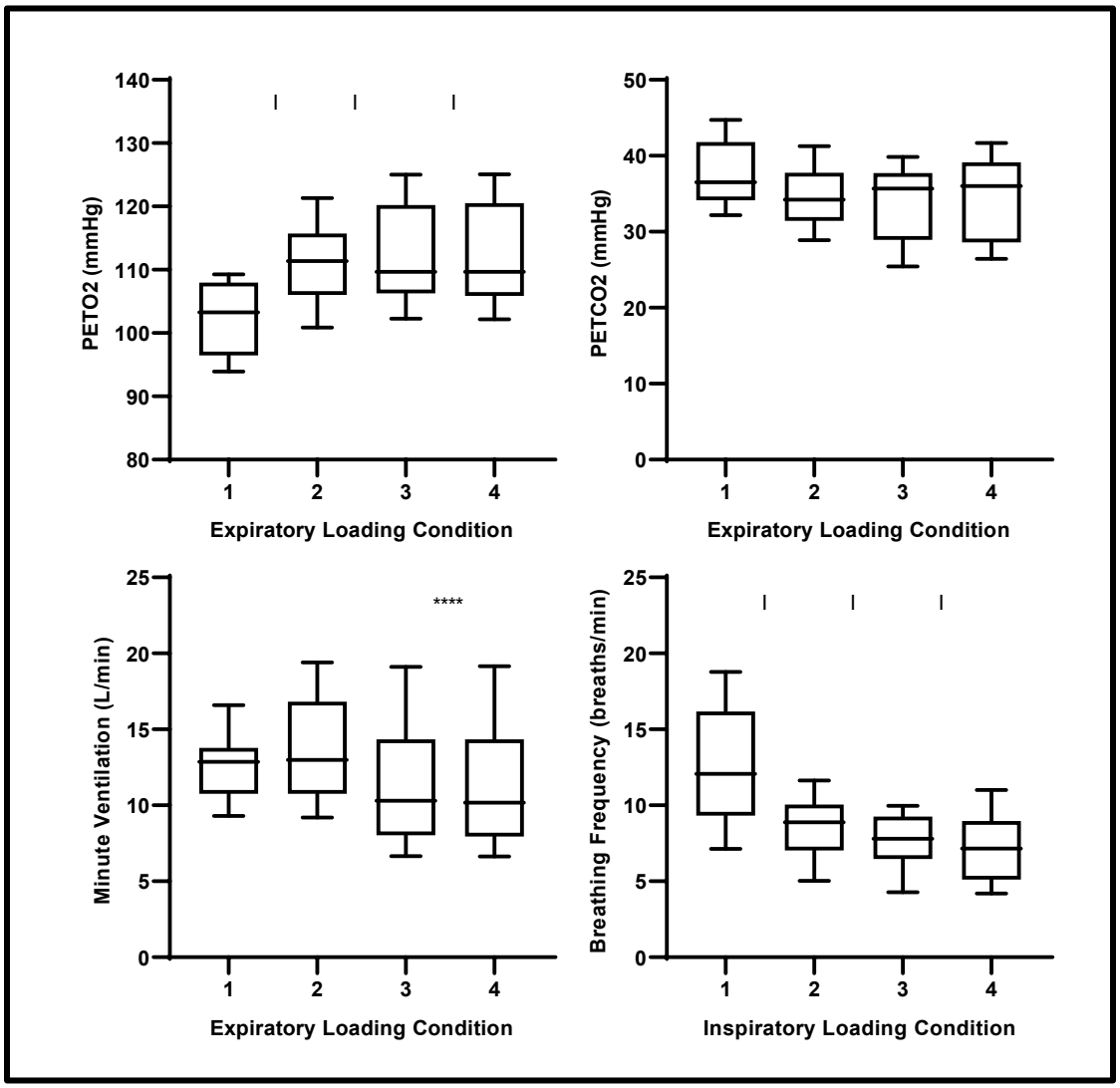


Figure 9. End-tidal gases and ventilatory responses to expiratory threshold loading during masked conjunctive continuous performance trials. Values represent means ± SEM. $P_{ET}O_2$ and $P_{ET}CO_2$: end-tidal partial pressures of O_2 and CO_2. **** Significant difference from previous load condition, $P < 0.001$, Ɨ Significant difference from load condition 1, $P < 0.05$.

4.2.2 Masked Conjunctive Continuous Performance Task Results During Expiratory Loading. There were observable changes in MCCPT performance across expiratory threshold loading conditions. Specifically, median hit RT was significantly longer during Loads 3 and 4 compared with Load 1 (**Table 3, Figure 10**; $P < 0.05$). However, there were no differences in the omission, nor the commission error rates observed between the expiratory loading conditions (**Figure 11**). Further there was no difference between loads for median hit RT when presented with color conjunctive and non-conjunctive distractor stimuli. There was, however, a significant difference in median hit RT between load 3 and load 1 when presented with a shape conjunctive distractor stimulus ($P = 0.01$). These data suggest high expiratory threshold loads may impact attentional processing in that the largest loads (i.e., loads 3 and 4) delayed processing and reaction times. In contrast to inspiratory threshold loaded breathing, large expiratory loads appear to have little influence on a participant's engagement and thus, the ability to discriminate between the target and distractor stimuli [22].

Table 3. Median Hit Reaction Times Across Expiratory Threshold Loading Conditions.

Loading Conditions			
Condition	**Mean**	**SD**	***p-value***
1 (No Load)	458.6	97.5	
2 (Light Load)	448.8	53.9	0.56
3 (Moderate Load)	467.9	56.0	**0.030**
4 (Heavy Load)	465.6	69.7	**0.047**

SD: standard deviation. Bolded *p-values* denote a significant difference between the inspiratory and expiratory load and load 1 ($P < 0.05$).

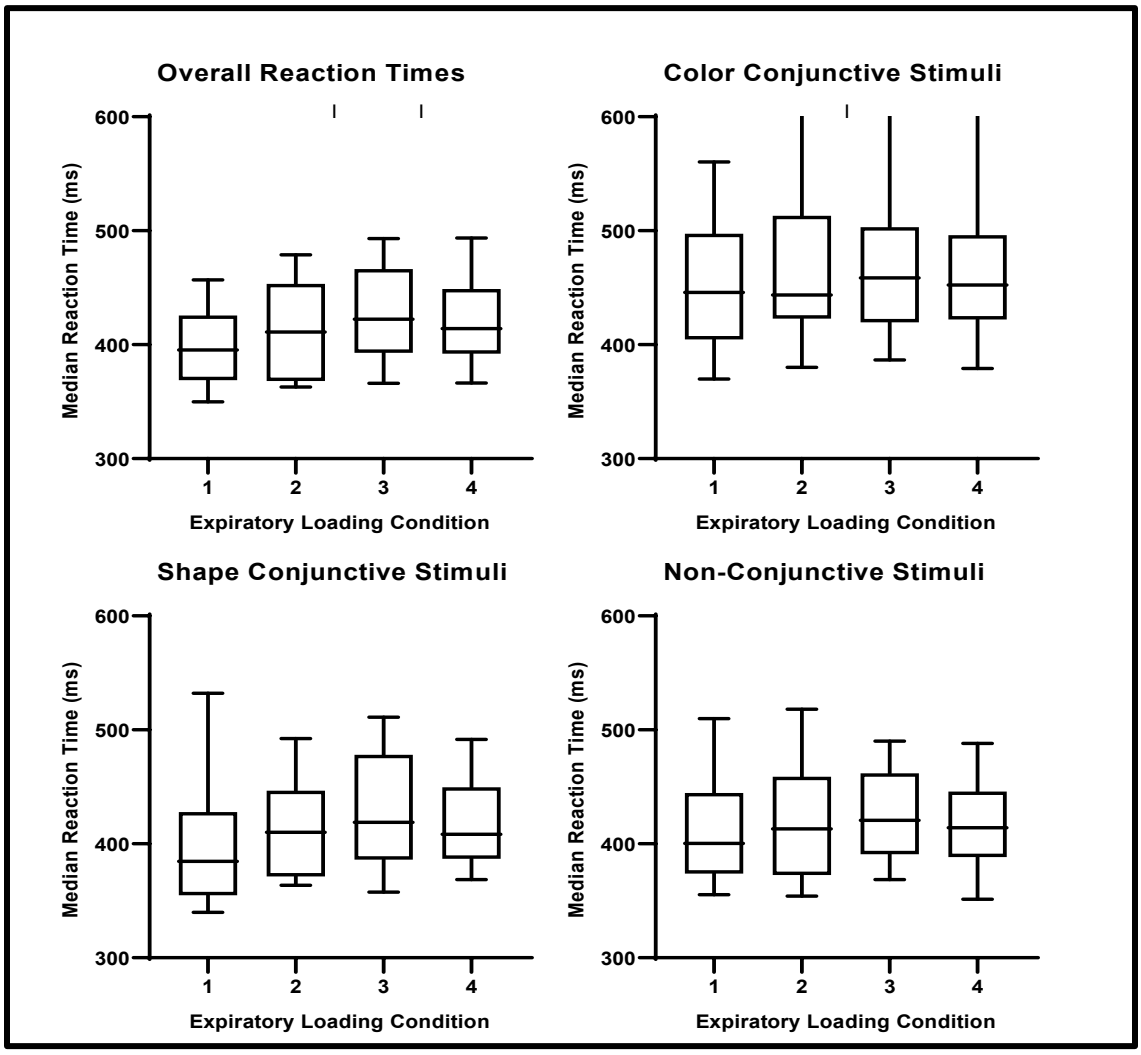


Figure 10. Median reactions times for different stimuli across inspiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. Ł Significant difference from load condition 1, $P < 0.05$.

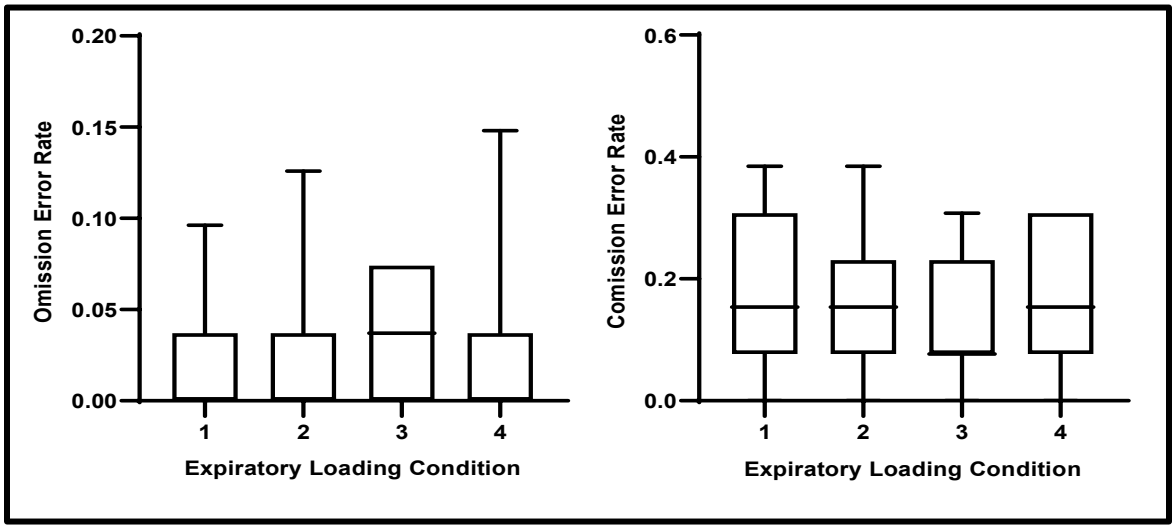


Figure 11. Error rates across expiratory threshold loading conditions during masked conjunctive continuous performance trials. Values represent means ± SEM. There were no differences in error rates between loading conditions.

4.3 Nonlinear Trends in Reaction Times

4.3.1 Inspiratory Threshold Loaded Breathing

4.3.1.1 Reaction Times During Inspiratory Threshold Loaded Breathing

Figure 12 illustrates the effect of inspiratory effort sensation on overall median hit RT. Increased inspiratory effort sensation had deleterious effects on the overall, color conjunctive, and shape conjunctive median hit reaction times (**Table 4** and **Supplementary Table 1**). Across all stimuli types (e.g., non-conjunctive, color conjunctive, etc.), as effort sensation increases, RT increased as well. Interestingly, there was no main effect of inspiratory PTP on overall median hit RT but demonstrated a negative association with color conjunctive and non-conjunctive stimuli. Furthermore, there were no main effects of $P_{ET}O_2$ or $P_{ET}CO_2$ for overall, color conjunctive, shape conjunctive, and non-conjunctive median hit RT.

These data demonstrate the perception of the inspiratory load is the primary factor influencing overall median hit RT, not the quantitative amount of power (i.e., effort) expended during inspiration. Specifically, as a participant's perception of their breathing effort increased, their central processing (i.e., RT) was delayed. This relationship between a participant's perception of breathing effort and delayed central processing was present across all stimuli types. These data support our hypothesis that imposing a "distraction" via increased respiratory effort sensation leads to impairments in cognitive performance, specifically within the domain of attentional focus. Thus, we believe the perception of breathing effort may indeed occupy a portion of attentional resources and increasing respiratory effort sensation, in addition to the strain of continuous engagement required by the MCCPT, may stress attentional resources to the point of delayed processing time.

Table 4. GAMM results for Overall Median Hit Reaction Time During Inspiratory Loaded Trials

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	6.07	0.02	283.30	**<0.001**
s(Effort Sensation)	0.79	-	6.65	**<0.001**
s(Pressure-Time Product)	<0.01	-	0.00	0.13
s(PETCO2)	<0.01	-	0.00	0.57
s(PETO2)	<0.01	-	0.00	0.16
Random Effects				
ID	0.11	-	99.63	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	**<0.05**
ID: Pressure-Time Product	<0.01	-	0.00	0.25
ID:PETCO2	<0.01	-	0.00	0.29
ID:PETO2	<0.01	-	0.00	0.29

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; PETCO2 and PETO2 were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

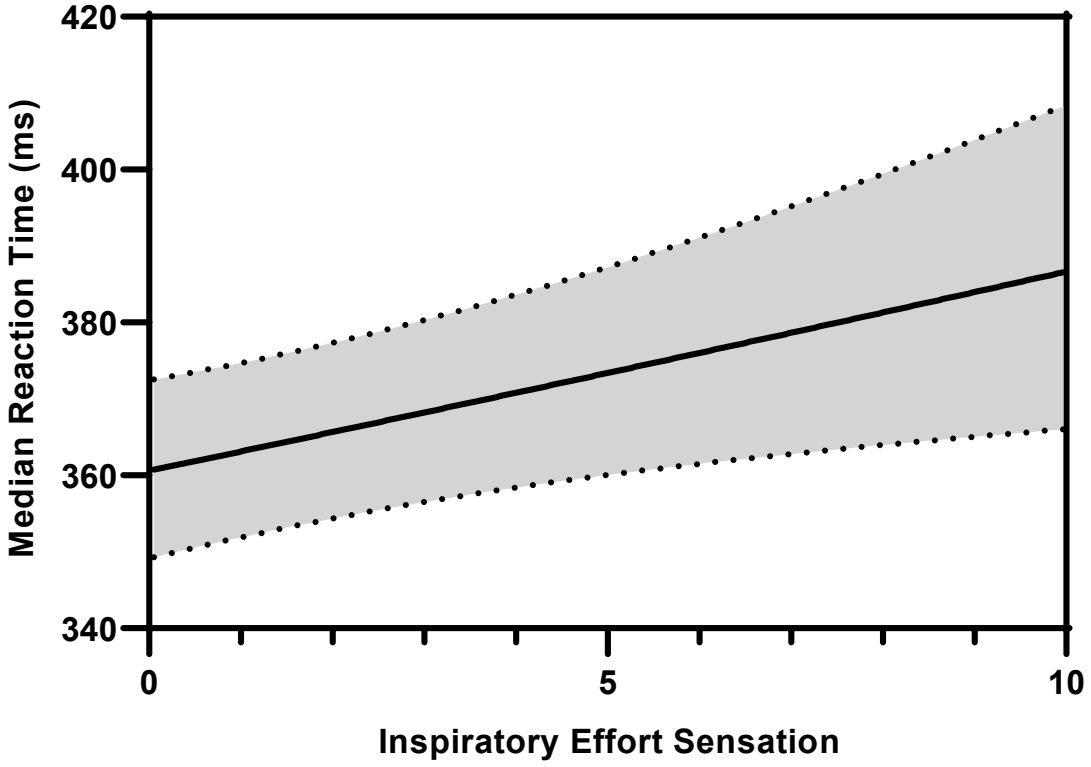


Figure 12. The influence of inspiratory effort sensation on median reaction times.

4.3.1.2 Error Rates During Inspiratory Threshold Loaded Breathing

Figure 13 illustrates the effect of inspiratory threshold loading on total error rates. There was no main effect of inspiratory effort sensation on total error rates (**Table 5**). Further, there were no main effects of pressure-time product or the change in $P_{ET}CO_2$ from baseline. On the other hand, there was a main effect of the percent change in $P_{ET}O_2$. These data suggest a small increase in $P_{ET}O_2$ (up to approximately a 10% increase from baseline) may improve total error rates but an increase in $P_{ET}O_2$ beyond that ~10% threshold increases error rates. Conversely, a decrease in $P_{ET}O_2$ from baseline is associated with an increase in total error rates.

When total error rates were stratified by error type, we observed a similar relationship between percent change in $P_{ET}O_2$ and commission errors (responding to a stimulus the subject was *not* supposed to) (**Supplementary Table 2**). Specifically, an increase or decrease in $P_{ET}O_2$ from the approximate baseline may improve commission error rates during inspiratory threshold loaded breathing. Further, there were no main effects of effort sensation, pressure-time product, or $P_{ET}CO_2$ on commission error rates. Additionally, there was a main effect of pressure-time product on omission error rates (not responding to a stimulus the subject *was* supposed to) (**Supplementary Table 2**). Taken together, these data demonstrate a small increase in $P_{ET}O_2$ ($\leq$10%) from baseline may induce a "hypervigilant state" wherein participants were more capable of maintaining continuous engagement in the task, as evidenced by improvements in target and distractor discrimination. However, further increase in $P_{ET}O_2$ had a deleterious effect on error rates, suggesting a small operationally relevant window of $P_{ET}O_2$ that should be maintained for optimal performance.

Table 5. GAMM results for Total Error Rates During Inspiratory Loaded Trials

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	– 0.93	0.11	– 8.52	**<0.001**
s(Effort Sensation)	<0.01	-	0.00	0.14
s(Pressure-Time Product)	<0.01	-	0.00	0.94
s($P_{ET}CO_2$)	<0.01	-	0.00	0.65
s($P_{ET}O_2$)	1.00	-	18.29	**<0.01**
Random Effects				
ID	0.10	-	526.47	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	0.13
ID: Pressure-Time Product	<0.01	-	0.00	0.79
ID: $P_{ET}CO_2$	<0.01	-	0.00	0.33
ID: $P_{ET}O_2$	<0.01	-	0.00	0.07

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

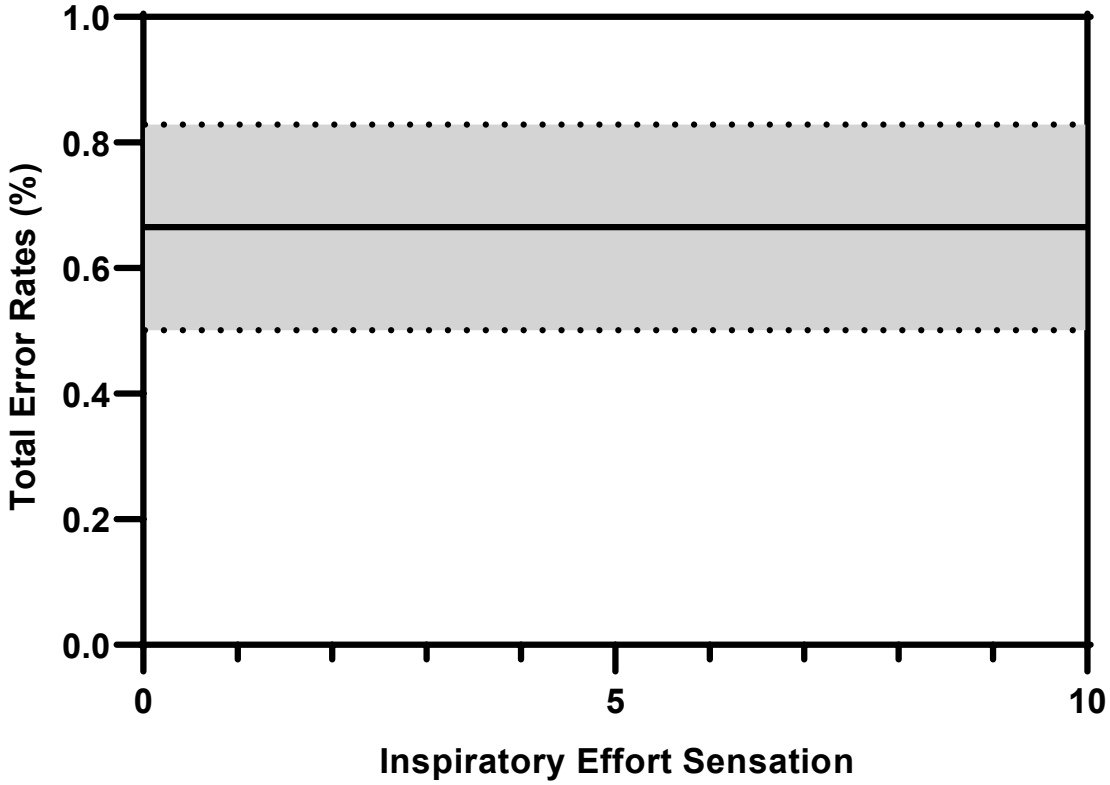


Figure 13. The influence of inspiratory effort sensation on total error rates.

4.3.2 Expiratory Threshold Loaded Breathing

4.3.2.1 Reaction Times During Expiratory Threshold Loaded Breathing

Figure 14 illustrates the effect of expiratory effort sensation on overall median hit RT. There was no main effect of expiratory effort sensation on overall median hit RT (**Table 6**). However, when RTs are stratified by stimuli type, there is a main effect of expiratory effort sensation on median hit RT for shape and non-conjunctive stimuli, but not for color conjunctive stimuli. Specifically, as expiratory effort sensation increases, median hit RT increases as well for both shape conjunctive and non-conjunctive stimuli (**Supplementary Table 3**). Similarly, shape conjunctive stimuli median RTs increase when expiratory effort sensation increased until an effort sensation rating of ~6 (i.e., strong to very strong), after which, median RTs decrease as effort sensation further increases.

We also observed a similar relationship between median hit RT and $P_{ET}CO_2$, wherein there was no main effect of $P_{ET}CO_2$ (**Table 6**) on overall median hit RT, yet there was a main effect of $P_{ET}CO_2$ on shape, color, and non-conjunctive stimuli (**Supplementary Table 3**). Indeed, as $P_{ET}CO_2$ decreases from baseline, median RT increases. On the other hand, as $P_{ET}CO_2$ increases from baseline, median RT decreases to become faster. These data suggest lower $P_{ET}CO_2$ is associated with higher median RTs while higher $P_{ET}CO_2$ is associated with lower median RTs.

Taken together, these data suggest high expiratory loads may not influence overall central processing speed to the degree that high inspiratory loads do but may exert its impact on performance in the way of decreasing engagement. This decreased engagement and reduced perception are demonstrated in the increased processing delay when presented with shape and non-conjunctive stimuli.

Table 6. GAMM results for Overall Median Hit Reaction Time During Expiratory Loaded Trials

	Estimate*	SE	Statistic	*p-value*
Main Effect				
Intercept	6.08	0.03	192.1	**<0.001**
s(Effort Sensation)	1.37	-	3.07	0.49
s(Pressure-Time Product)	0.43	-	5.63	0.63
s($P_{ET}CO_2$)	0.62	-	10.22	0.31
s($P_{ET}O_2$)	<0.01	-	0.00	0.69
Random Effects		-		
ID	9.64		391.57	**<0.001**
ID:Effort Sensation	5.68	-	157.17	**<0.05**
ID:Pressure-Time Product	<0.01	-	0.00	**<0.05**
ID:$P_{ET}CO_2$	<0.01	-	0.00	**<0.05**
ID:$P_{ET}O_2$	<0.01	-	0.00	**<0.001**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

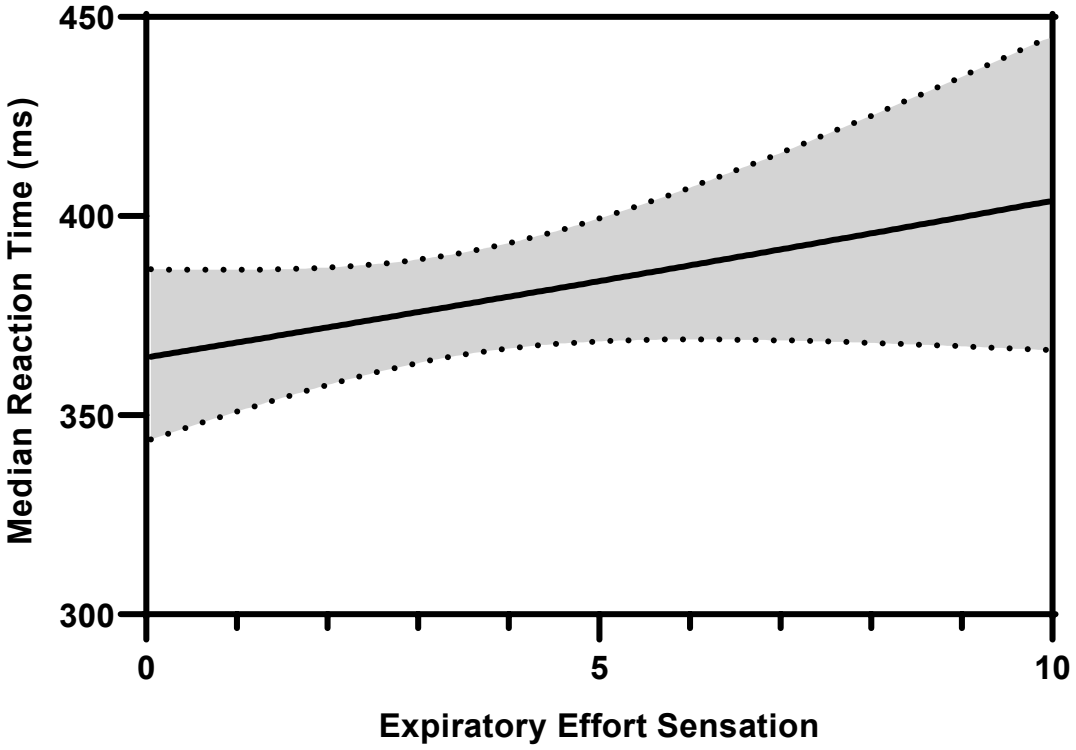


Figure 14. The influence of expiratory effort sensation on median reaction times.

4.3.2.2 Error Rates During Expiratory Threshold Loaded Breathing

Figure 15 illustrates the effect of expiratory threshold loading on total error rates. There were no main effects of expiratory effort sensation, pressure-time product, or percent change in $P_{ET}CO_2$ nor $P_{ET}O_2$ on total error rates (**Table 7**). However, when total error rates were stratified by error type, we observed a significant main effect of percent change in $P_{ET}CO_2$ on commission error rates (**Supplementary Table 4**). Specifically, any deviation in $P_{ET}CO_2$, whether above or below baseline, is associated with a decrease in commission error rates. Interestingly, an increase in effort sensation was associated with decreased commission error rates. Conversely, there was a main effect of effort sensation on omission error rates wherein we observed a significant positive relationship between effort sensation and omission error rates. It must be noted that decreased commission error rates and increased omission error rates are both product of non-responses, in that not responding to the target stimuli would increase omission errors and not responding to the distractor stimuli (i.e., red circle) would improve commission errors. As such, these results may suggest a global reduction in engagement.

Table 7. GAMM results for Total Error Rates During Expiratory Loaded Trials

	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	– 0.81	0.12	– 6.69	**<0.001**
s(Effort Sensation)	<0.01	-	0.00	0.49
s(Pressure-Time Product)	<0.01	-	0.00	0.63
s($P_{ET}CO_2$)	<0.01	-	0.00	0.31
s($P_{ET}O_2$)	<0.01	-	0.00	0.69
Random Effects		-		
ID	9.11		1340.2	**<0.001**
ID:Effort Sensation	5.13	-	641.7	**<0.001**
ID:Pressure-Time Product	<0.01	-	0.00	**<0.05**
ID:$P_{ET}CO_2$	<0.01	-	0.00	**<0.05**
ID:$P_{ET}O_2$	<0.01	-	0.00	**<0.05**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

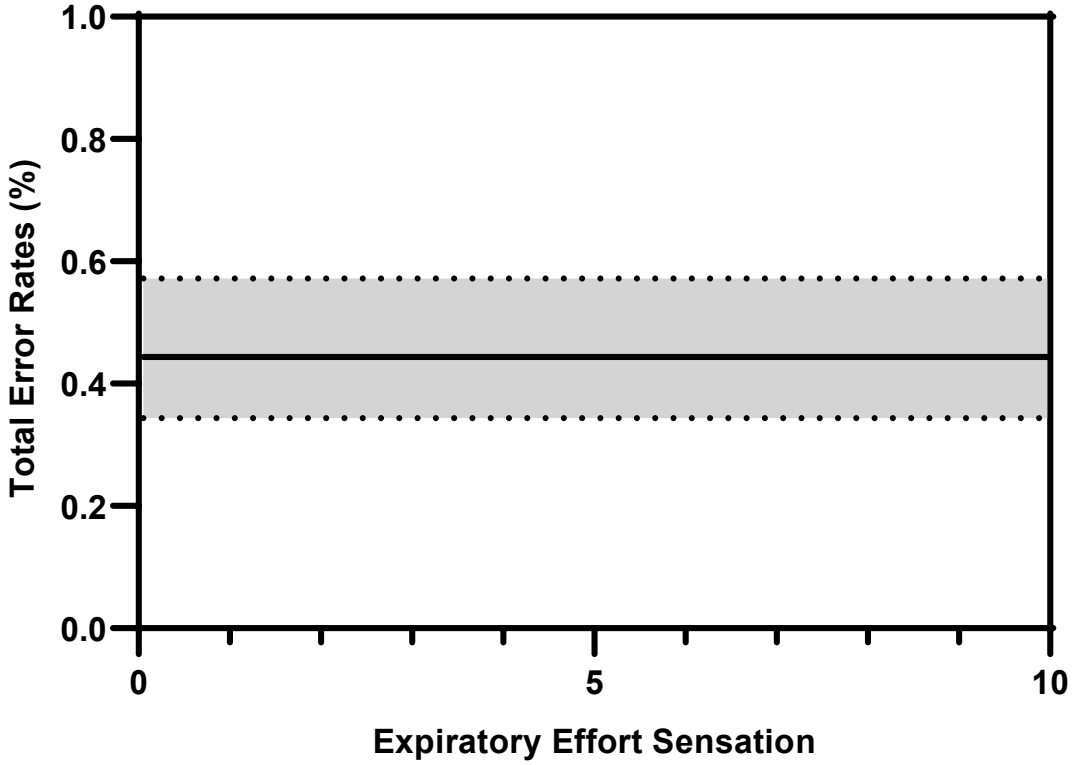


Figure 15. The influence of expiratory effort sensation on total error rates.

4.4 Further Considerations

It is apparent from our data that by imposing progressively larger inspiratory and expiratory threshold loads during spontaneous breathing, central processing was systematically delayed (**Figures 5** and **10**). However, through interrogation of non-linear GAMM modelling, the level of inspiratory and expiratory load did not have an effect of central processing time when controlling for the other covariates in the model; rather, it appears respiratory effort sensation was the primary effector of central processing time (**Tables 5** and **7).** Stated in other words, the observed differences in RT were influenced by the participant's perception of breathing effort, independent of the actual load applied. Additionally, our cohort demonstrated inter-participant variability, for which there are several factors which may have contributed to this variability in the present work. Firstly, as mentioned earlier, no feedback on breathing pattern or pressure-development was given to the participants and, as such, the ventilatory responses during the MCCPT trials and loading conditions were spontaneous. As such, participants were not obliged to maintain a specified respiratory pattern. Indeed, participants may have engaged in altered ventilatory patterns in an attempt to "avoid" the large expiratory loads (see Section 4.2.1). Secondly, it is known that simple RT is shorter during expiration than inspiration [25]. Given that stimulus presentations during the MCCPT trials were not standardized to any specific point of the respiratory cycle, a source of RT variability may have been introduced due to this phasic modulation of central processing time.

Moving forward, we propose the following three suggestions to pre-emptively lessen the burden of respiratory load on cognitive performance: 1) develop a screening protocol to assess pilots that tolerate and/or manage respiratory loads with minimal effects on RT; 2) develop a training program to progressively expose pilots to respiratory loads to train them to more effectively navigate the load; and 3) re-evaluate the threshold for acceptable system impedance in systems

requirements and deliver new systems to eliminate/minimize respiratory loads. To the second point, increasing global respiratory muscle strength would mean a given load would constitute smaller percentage of maximal respiratory strength. Thus, that given load may have less impact on cognitive performance by virtue of a lower respiratory effort sensation. An example of this last point is that the current discrete maximum recommended system impedance may still impose respiratory loads sufficient to deleteriously influence cognitive performance. As such, it may be imperative to set a threshold for acceptable system impedance below the current standards to optimize pilot performance.

Additionally, we offer the following three suggestions to more effectively assess the effects of inspiratory/expiratory loading on central processing: 1) randomized load timing and intensities so the participant is unable to anticipate and therefore modulate their respiratory pattern to account for the load, which may strain central processing to a further degree than constant loading; 2) physical exertion can be added as an additional perturbation during loaded breathing; 3) investigate the influence of an imposed respiratory load on cognitive performance under more operational conditions. An example of this last point is found in McMorris *et al.* [26, 27], wherein these investigators had participants perform exercise at varying intensities while performing a simple RT test. These studies, as well as other previous literature, suggest that RT generally decreases (i.e., becomes faster) with moderately intense exercise, but increases (i.e., becomes slower) at more intense levels of physical exertion [26-30]. Utilizing exercise as a modality to stress central processing may have operational relevancy to pilots who often undergo significant physical stress in the form of anti-G straining maneuvers. As such, exercise intensity may occupy a portion of central processing, in addition to respiratory effort sensation, and the addition of physical stress may further delay central processing time and increase error-rate.

4.5 Implications of Findings

The most robust finding of the present work was that median hit RT was positively associated with respiratory effort sensation during inspiratory and expiratory threshold loading (**Figures 12** and **14**). According to the smoothed estimates of the GAMM model for this relationship, one could expect that median RT would increase approximately 26ms and 41ms as inspiratory and expiratory effort sensation rises from 0 to 10 respectively. It is important to note that these increases in RT constitute delays in processing and reaction for a single task. From an operational perspective, a 120-minute flight where the pilot performs 10 tasks/min constitutes 1,200 total tasks during flight. Were a pilot's central processing delayed by an average of 20ms, the total reaction time delay during this 120-minute flight would be ~24sec, which may prove operationally-relevant. These data demonstrate higher respiratory effort sensation during expiratory threshold loaded trial had a larger effect (i.e., more negative) on median RT when compared with inspiratory threshold loading. As a result, participants may have adopted altered respiratory patterns to avoid the load and minimize respiratory effort sensation, a finding consistent with previous literature [23, 24]. However, this respiratory pattern may alter $P_{ET}CO_2$. It is important to note that while hyper- and hypocapnia have been associated with altered cognitive performance, there are conflicting reports as to their relationship (i.e., positive or negative) [14-16]. While there was no association between $P_{ET}CO_2$ and median RT during inspiratory loaded trials, our data did demonstrate a parabolic relationship between $P_{ET}CO_2$ and median RT during expiratory loaded trials. Specifically, lower $P_{ET}CO_2$ is associated with higher median RTs while higher $P_{ET}CO_2$ is associated with lower median RTs. Taken together, the current data demonstrates minimizing respiratory effort sensation and maintaining end-tidal gas concentrations relative to baseline during flight may prove useful in reducing pilot RTs during complex behavioral tasks.

5.0 CONCLUSIONS

The present work examined the effects of inspiratory and expiratory threshold loading on attentional performance, as measured via a modified version of the MCCPT. Our findings demonstrate that augmenting respiratory effort sensation may delay central processing. To this end, increasing either inspiratory or expiratory effort sensation, independent of actual respiratory load, may prolong RT. These findings are in accordance with our previous findings detailed in the DoD/USAF-funded project entitled "The Cognitive Performance Cost of Increased Work of Breathing" (Task Order: 0052; Grant number: FA865012D6280). The current study demonstrates that when high inspiratory threshold loads were imposed, participants had difficulty identifying the target and discriminating it from the distractors. These findings support our hypothesis, in that breathing on high inspiratory loads may stress attentional resources preventing the participants from continuously engaging in the MCCPT, thus diminishing perception and delaying RT [22].

Additionally, the data demonstrated an influence of $P_{ET}O_2$ on error rates during inspiratory threshold loading trials. Specifically, we demonstrated a small increase in $P_{ET}O_2$ from baseline may induce a "hypervigilant state" wherein participants were more capable of maintaining continuous engagement in the task. This is supported by the improvements in target and distractor discrimination. However, a >10% increase or decrease in $P_{ET}O_2$ from baseline was associated with increased error rates. These data suggest a small operationally relevant window of $P_{ET}O_2$ that should be maintained for optimal performance. Interestingly, inspiratory effort sensation did not demonstrate an influence on error rates. These data suggest alterations in end-tidal gases may modulate brain blood flow and affect one's ability to discriminate between the "target" and "non-target" stimuli, resulting in increased error rates [31, 32].

These data support our hypothesis that imposing a "distraction" via increased respiratory effort sensation leads to impairments in cognitive performance within the domain of attentional focus. Thus, we believe the perception of breathing effort may indeed occupy a portion of attentional resources and increasing respiratory effort sensation may stress attentional resources to the point of delayed processing time. As such, it is reasonable that minimizing respiratory effort sensation (independent of device resistance) and maintaining end-tidal gas concentrations relative to baseline during flight operations may prove useful in reducing pilot RTs during complex behavioral tasks.

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APPENDIX A – Supplementary GAMM Results Tables for Inspiratory Loading Conditions

Supplementary Table 1. GAMM results for Median Hit Reaction Time for Color, Shape, and Non-Conjunctive Stimuli During Inspiratory Loaded Trials

Color Conjunctive Stimuli				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	6.18	0.02	368.70	**<0.001**
s(Effort Sensation)	0.54	-	0.59	**<0.05**
s(Pressure-Time Product)	0.25	-	0.45	**<0.01**
s($P_{ET}CO_2$)	0.44	-	0.79	0.03
s($P_{ET}O_2$)	<0.01	-	0.00	0.41
Random Effects				
ID	9.63	-	22.93	**<0.001**
ID:Effort Sensation	<0.01	-	1.36	0.09
ID:Pressure-Time Product	<0.01	-	0.00	0.34
ID:$P_{ET}CO_2$	<0.01	-	0.00	**<0.01**
ID:$P_{ET}O_2$	<0.01	-	0.00	**<0.001**
Shape Conjunctive Stimuli				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	6.06	0.02	287	**<0.001**
s(Effort Sensation)	0.72	-	2.34	**<0.01**
s(Pressure-Time Product)	<0.01	-	2.47	0.21
s($P_{ET}CO_2$)	<0.01	-	0.00	0.69
s($P_{ET}O_2$)	<0.01	-	0.00	0.24
Random Effects				
ID	0.10	-	52.79	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	**<0.05**
ID:Pressure-Time Product	<0.01	-	0.00	0.21
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.24
ID:$P_{ET}O_2$	<0.01	-	0.00	0.69
Non-Conjunctive Stimuli				
	Estimate*	SE	T-value	*p-value*
Main Effects				
Intercept	6.06	0.02	296.40	**<0.001**
s(Effort Sensation)	<0.01	-	0.00	0.14
s(Pressure-Time Product)	1.07	-	9.14	**<0.05**
s($P_{ET}CO_2$)	<0.01	-	0.00	0.79
s($P_{ET}O_2$)	<0.01	-	0.00	0.48
Random Effects		-		
ID	0.10		52.32	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	0.14
ID:Pressure-Time Product	<0.01	-	0.00	0.42
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.89
ID $P_{ET}O_2$	<0.01	-	0.00	0.78

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Supplementary Table 2. GAMM results for Commission and Omission Errors During Inspiratory Loaded Trials

Commission Errors				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	-1.10	0.12	-9.35	**<0.001**
s(Effort Sensation)	<0.01	-	0.00	0.25
s(Pressure-Time Product)	<0.01	-	0.00	0.55
s($P_{ET}CO_2$)	<0.01	-	0.00	0.39
s($P_{ET}O_2$)	0.97	-	21.36	**<0.01**
Random Effects				
ID	0.11	-	763.21	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	0.25
ID:Pressure-Time Product	<0.01	-	0.00	0.39
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.26
ID:$P_{ET}O_2$	<0.01	-	0.00	0.24
Omission Errors				
	Estimate*	SE	T-value	*p-value*
Main Effects				
Intercept	6.06	0.02	296.40	**<0.001**
s(Effort Sensation)	<0.01	-	0.00	0.14
s(Pressure-Time Product)	1.07	-	9.14	**<0.05**
s($P_{ET}CO_2$)	<0.01	-	0.00	0.79
s($P_{ET}O_2$)	<0.01	-	0.00	0.48
Random Effects				
ID	0.10	-	52.32	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	0.14
ID:Pressure-Time Product	<0.01	-	0.00	0.42
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.89
ID:$P_{ET}O_2$	<0.01	-	0.00	0.78

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

APPENDIX B – Supplementary GAMM Results Tables for Expiratory Loading Conditions

Supplementary Table 3. GAMM results for Median Hit Reaction Time for Color, Shape, and Non-Conjunctive Stimuli During Expiratory Loaded Trials

Color Conjunctive Stimuli				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	6.14	0.03	244.6	**<0.001**
s(Effort Sensation)	0.23	-	0.12	0.07
s(Pressure-Time Product)	0.01	-	0.00	0.07
s($P_{ET}CO_2$)	0.79	-	13.52	**<0.001**
s($P_{ET}O_2$)	<0.01	-	0.00	0.07
Random Effects				
ID	8.10	-	162.03	**<0.001**
ID:Effort Sensation	4.94	-	42.96	**<0.001**
ID:Pressure-Time Product	2.02	-	30.59	**<0.05**
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.07
ID:$P_{ET}O_2$	<0.01	-	0.00	0.12
Shape Conjunctive Stimuli				
	Estimate*	SE	T-value	*p-value*
Main Effects				
Intercept	6.08	0.04	168.4	**<0.001**
s(Effort Sensation)	1.53	-	1.99	**<0.001**
s(Pressure-Time Product)	<0.01	-	0.00	0.35
s($P_{ET}CO_2$)	1.38	-	13.81	**<0.001**
s($P_{ET}O_2$)	<0.01	-	0.00	0.35
Random Effects				
ID	9.76	-	94.28	**<0.001**
ID:Effort Sensation	<0.01	-	0.00	**<0.05**
ID:Pressure-Time Product	<0.01	-	0.00	**<0.05**
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.16
ID:$P_{ET}O_2$	<0.01	-	0.00	0.16
Non-Conjunctive Stimuli				
	Estimate*	SE	T-value	*p-value*
Main Effects				
Intercept	6.07	0.03	197	**<0.001**
s(Effort Sensation)	0.72	-	2.81	**<0.01**
s(Pressure-Time Product)	<0.01	-	0.00	0.41
s($P_{ET}CO_2$)	0.73	-	10.99	**<0.01**
s($P_{ET}O_2$)	<0.01	-	0.00	0.75
Random Effects				
ID	9.48	-	195.77	**<0.001**
ID:Effort Sensation	<0.01	-	52.51	**<0.001**
ID:Pressure-Time Product	<0.01	-	0.00	**<0.05**
ID:$P_{ET}CO_2$	<0.01	-	0.00	**<0.01**
ID:$P_{ET}O_2$	<0.01	-	0.00	**<0.05**

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

Supplementary Table 4. GAMM results for Commission Errors During Expiratory Loaded Trials

Commission Errors				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	-1.07	0.10	-10.87	**<0.001**
s(Effort Sensation)	0.47	-	3.45	0.06
s(Pressure-Time Product)	<0.01	-	0.00	0.79
s($P_{ET}CO_2$)	1.38	-	70.51	**<0.01**
s($P_{ET}O_2$)	<0.01	-	0.00	0.27
Random Effects				
ID	9.02	-	513.14	**<0.001**
ID:Effort Sensation	<0.01	-	50.53	**<0.01**
ID:Pressure-Time Product	<0.01	-	0.00	0.13
ID:$P_{ET}CO_2$	<0.01	-	0.00	0.41
ID:$P_{ET}O_2$	<0.01	-	0.00	0.20
Omission Errors				
	Estimate*	SE	Statistic	*p-value*
Main Effects				
Intercept	-1.81	0.08	-21.72	**<0.001**
s(Effort Sensation)	0.83	-	96.77	**<0.001**
s(Pressure-Time Product)	<0.01	-	0.00	0.94
s($P_{ET}CO_2$)	<0.01	-	0.00	0.11
s($P_{ET}O_2$)	<0.01	-	0.00	0.77
Random Effects				
ID	5.45	-	2536.77	**<0.001**
ID:Effort Sensation	3.34	-	444.37	**<0.001**
ID:Pressure-Time Product	4.94	-	2743.20	**<0.001**
ID:$P_{ET}CO_2$	1.00	-	83.44	**<0.001**
ID:$P_{ET}O_2$	<0.01	-	0.00	0.10

SE: standard error; Statistic refers to the T-value for the intercept and the F-value for the smooth terms and random effects; Pressure-time product was measured in cmH_2O*min; $P_{ET}CO_2$ and $P_{ET}O_2$ were measured in mmHg; Bolded *p-values* denote a significant influence of the covariate term on overall median hit reaction times (P <0.05). *For all smooth terms (s()) this estimate represents the estimated degrees of freedom of the corresponding smooth.

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
AIC	Akaike information criterion
CO2	Carbon-dioxide
MCCPT	Masked Conjunctive Continuous performance task
CR10	10-point Category ratio scale
Δ	Change score
ES	Muscular effort sensation rated on the 10-point Category ratio scale
FEV1	Forced expiratory volume in 1 s
FIF25%	Forced inspiratory flow at 25% of expired volume
FIF25-75%	Average forced inspiratory flow across the midexpiratory volumes
FIF50%	Forced inspiratory flow at 50% of expired volume
FIF75%	Forced inspiratory flow at 75% of expired volume
FIFmax	Maximal forced inspiratory flow
FVC	Forced vital capacity
GAMM	Generalized additive mixed effects model
IC	Inspiratory capacity
MEP	Maximal expiratory mouth pressure
MIP	Maximal inspiratory mouth pressure
NEDU	Navy Experimental Diving Unit
NS	Not significant
n	Number of subjects
Pe_{avg}	Average expiratory pressure
Pi_{avg}	Average inspiratory pressure
Pb	Power of breathing
PTP	Pressure-time product
RT	Reaction time
T_e	Expiratory time
T_i	Inspiratory time
Wb	Work of breathing

TASK 3: POTENTIAL CAUSES OF DECREMENTS IN LUNG PHYSIOLOGY IN HIGH PERFORMANCE FLIGHT OPERATIONS

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1.0 SUMMARY

Task 3 ultimately was designed around two separate studies. The first study was focused on inspiratory loading to the point of task failure or some evidence of respiratory muscle fatigue to determine if sequential 30-minute windows of loaded breathing would trigger or result in any decrements in lung physiology. The second project was focused on the interaction between ventilator induced breathing in the presence of a relatively severe elastic chest wall load. For the second project, the premise was to determine if positive pressure breathing in the presence of reduced ability for lung expansion would result in any risks of altering lung fluid status or cause airway inflammation. These two projects are presented as two separate reports sequentially. These were pursued through the following general aims.

Task 3. Potential <u>Causes of Decrements in Lung Physiology in High Performance Flight Operations (15-18 months)</u>

<u>Aim 1.</u> To determine the influence of graded inspiratory pressure threshold loads (degree and duration of exposure) on measures of decrements in lung physiology in healthy adults. *We hypothesize that maximal threshold will cause evidence of lung inflammation and deterioration in lung function that will be more significant with higher levels of inspiratory threshold loading and/or when exposures are repeated.*

<u>Aim 2.</u> To determine the influence of positive pressure breathing with small elastic load on measures of decrement in lung physiology in healthy. *We hypothesize that positive pressure breathing will cause evidence of lung inflammation and deterioration in lung function that will be more significant with higher levels positive pressure breathing and/or when exposures are repeated.*

2.0 INTRODUCTION

Although, pilots are exposed to many stressors while inflight, the Department of Defense (DoD) has decreased the number of physiological episodes and hypoxic events by more than half in 2019 after initiating a series of changes targeted at decreasing physiological incidents. However, pilots are exposed to a number of stressors during high performance flight operations. [1-3] Consequently, this intuitive was unable to eradicate the occurrence of physiological episodes and hypoxic symptoms altogether. As a result, of the continued physiological episodes and hypoxic like symptoms the pilots were experiencing a scientific task force was developed. The scientific task force began to examine the life support systems of the aircrafts to determine if there were any vulnerabilities to the pilots. Research completed by the Restrictive Breathing Working Group (RBWG) found that the on-board oxygen delivery system (OBOGS) caused at times a dramatic increase in the work of breathing for pilots when flying.[4] Early stages of inquiry into the physiological incidents with military aircrafts led to providing pilots with a maximum inspired oxygen concentration at lower altitudes. The pressure generated by the OBOGS provides resistance that pilots had to overcome, thus increasing the work of breathing. Increased work of breathing can result in pilots hyper or hypoventilating to compensate for the increased respiratory work and may also distract pilots from operational tasks.[5]

Modern aircrafts use an OBOGS to provide breathing air to the pilots, however, this exposes the pilots to high levels of oxygen (O_2), which can be toxic to cells and cause inflammation. The high concentrations of O_2 provided by the OBOGS with the rapid decline in nitrogen can result in the alveoli collapsing leading to atelectasis as nitrogen levels decline (absorption atelectasis) and ventilation perfusion inhomogeneities. The lungs lie central to the interface between external forces, countermeasures, and maintenance of normal physiology. Thus,

this environment leads to a high work and cost of breathing that can result in decrements in lung physiology and alterations in lung fluid regulation. [6, 7]

The OBOGS are in place to provide positive airway pressure support. Currently, there is potential for mismatches in pressure support relative to demand which can result in higher than normal inflation pressures caused by alveolar overdistension or forms of atelectrauma due to high shear forces and biotrauma in conjunction with pro-micro injurious inflammatory responses. [7] These forms of microinjury are accompanied by disturbances in both the epithelial and endothelial barriers, leading to changes within the blood-gas barrier, resulting in potential shifts in lung fluid balance. While the majority of mechanical ventilatory related injuries occur in association with other physiological insults in the clinical setting; within healthy populations utilizing the positive pressure oxygen delivery systems has also been associated with additional stressors that may contribute to changes in lung physiology. [6]

Moreover, ventilation and perfusion mismatching can result in poorly oxygenated blood, altered cerebral blood flow and potentially cerebral hypoxia. Heavy, acute and chronic inspiratory loads such as the ones generated by the OBOGS can lead to changes in lung fluid balance and a deterioration in lung physiology (e.g., negative pressure pulmonary edema).[8] Heavy mechanical loads during breathing may also alter pressure gradients across the lungs as well as influence pulmonary vascular volumes for gas exchange. Therefore, it is imperative that the OBOGS is working correctly and is synchronized with the pilot. [9] Countermeasures are in place to limit the magnitude of positive pressure breathing, however, this is accompanied with elastic loads caused by strapping or upper pressure garments which may limit the ability to take a full inflation to reverse atelectasis and prevent over inflation. The upper pressure garments (elastic load) and timing of OBOGS can result in transient increases in the work of breathing (threshold loads),

mismatches in respiratory effort as well as airway and alveolar pressures (both positive and negative). The load around the thorax may obviate typical means to reverse the atelectasis, possibly leading to further heterogeneous mismatches in the load being presented to the lung tissue.

The upper pressure garments, along with other garments (Anit-G suits) aid in improving venous return and helps to direct arterial blood flow to the head. [10, 11] The upper pressure garments alone, in non-flight situations, can cause thoracic restriction, similar to that often achieved through the use of chest wall strapping. Chest wall strapping has been used in healthy subjects to imitate thoracic restriction associated with clinical syndromes. In healthy subjects, chest wall strapping has resulted in a 20-30% decrease in peak exercise capacity, impaired stroke volume and increased dyspnea intensity. [12, 13]. For pilots of high-performance aircraft, these upper pressure garments are utilized to work cohesively with the onboard ventilation system to maintain compliance of the lung-chest wall during high stress maneuvers.

The type and degree of respiratory load that leads to detriments in lung function is currently not clear. Strenuous contractions of the inspiratory muscles lead to large negative changes in intrathoracic pressure which can result in mechanical damage to the lungs.[14] Simulated air combat maneuvers may lead to respiratory muscle fatigue and increased inspiratory work during the flight maneuvers [15]. Researchers have found that after the completion of simulated air combat maneuvers elastance of the lungs continues to increase.[15] The purpose of this task was twofold. We set out to understand the effects of increases in the work of breathing as well as positive pressure on lung physiology in healthy adults. The purpose of first aim of this study was to determine the influence of inspiratory pressure threshold loads (degree and duration of exposure) on lung physiology in healthy adults. We hypothesized breathing against heavy inspiratory loads over 30-90 minutes would result in evidence of lung inflammation and

deterioration in lung function that would be more significant with higher inspiratory threshold loads. The purpose of the second aim of this study was to determine the influence of positive pressure breathing with a significant elastic load on measures of lung physiology in healthy individuals. We hypothesized that positive pressure breathing would result in evidence of lung inflammation and deterioration in lung physiology measures that would be more significant with higher levels of positive pressure breathing and/or when exposures are sequentially repeated.

3.0 METHODS, ASSUMPTIONS, PROCEDURES

3.1 Study Aim 1 Participants

A total of 24 male and female participants (Age: 27±7, BMI: 23.4±3.6, Female: 54%, (**Table 1**) were recruited and retained for arm 1. To be considered for the study subjects had to be between the ages of 18 and 50 with a body mass index (BMI) less than 30 kg/m^2 and be in good health. We excluded patients that had a history of anemia, and known cardiac, pulmonary, or metabolic disease. We further excluded patients that were active smokers within the last 6 months and had a 5 or more pack-years smoking history. The present study was approved by the Mayo Clinic Internal Review Board and all subjects signed informed consent prior to participation.

Table 1. Subject demographics for arm 1. Mean ± SD

	All	40%	60%
n	24	12	12
Female	13 (54%)	6 (50%)	7 (58%)
Age (years)	27±7 (19-40)	26±8	27±6
Height (cm)	172±10	174±10	170±9
Weight (kg)	70±14	75±18	65±10
BMI (kg/m^2)	23.4±3.6	24.3±3.5	22.4±3.5
FVC (% Predicted)	100±10	99±14	100±6
FEV_1 (% Predicted)	101±10	101±14	102±7
FEV_1/FVC	88±8	24.3±3.8	22.4±3.8
MIPS (cm H_2O)	104±27	101±27	107±27
MEPS (cm H_2O)	131±43	128±43	134±46
Hemoglobin (g/dL)	14.1±1.6	14.5±1.4	13.8±1.7
Hematocrit (%)	42.5±3.7	43.2±3.4	41.7±4.0

3.1.1 Experimental Design Study Aim 1

Subjects visited the laboratory on two separate occasions, visit one and visit two (**Figure 1**). Visit one consisted of baseline measures and lasted about 1 hour. Visit two was completed in 3.5 hours and consisted of sequential inspiratory loaded breathing and measures of lung physiology. All patients were randomized to an inspiratory loading group of either 40 or 60% of their mean inspiratory pressure (MIPS) after visit 1. In a seated position subjects were instructed to breathe against a threshold inspiratory load until they were no longer able to achieve the target pressure or for 30 minutes. Inspiratory loaded breathing was completed over three sequential bouts.

Following each bout of inspiratory loaded breathing, measures of lung physiology were completed (post 1, post 2, post 3). A description of methods and procedures for each visit is provided below.

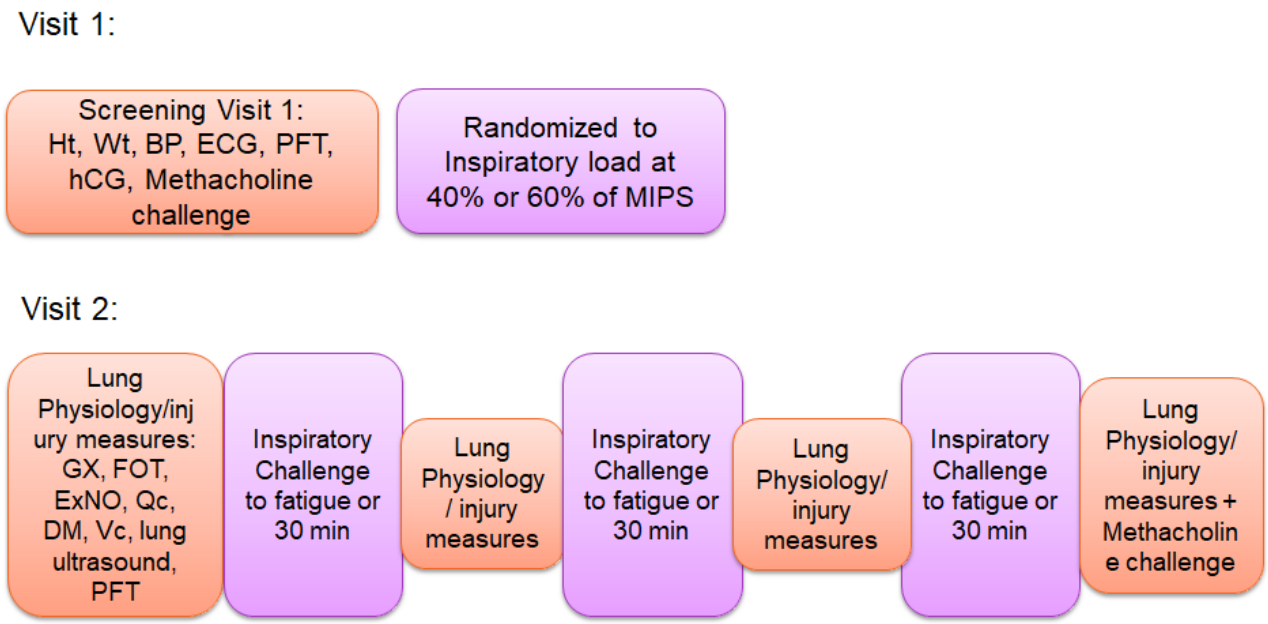


Figure 1. Study Aim 1 Protocol

3.1.2 Visit 1 - Familiarization Study Aim 1

During this visit subjects signed the informed consent and were randomized to one of two inspiratory load groups. Subjects past medical history, height, weight, blood pressure and heart rate were recorded. Subjects performed a pulmonary function test (PFT) which consisted of measuring slow vital capacity (SVC), forced vital capacity (FVC), and maximal voluntary ventilation (MVV). The original study design includes performing a methacholine challenge at Visit 1 and the end of Visit 2, but due to transient clinical practice modifications associated with the COVID pandemic, we were unable to perform the methacholine challenge in 18 of the 24 participants.

3.1.3 Visit 2 – Inspiratory challenge Study Aim 1

In a seated position, participants breathed on a Powerbreathe Plus (POWERbreathe International Ltd, Warwickshire, UK, **Figure 2**) which provided with a threshold inspiratory load set to either 40 or 60% of the subjects MIPS to fatigue or for 30 minutes at a rate of 15 breaths per minute and an inspiratory time to total breath time (T_I/T_{Tot}) of 60%, queued by a metronome. Each subject completed three sequential rounds of inspiratory loading. The first four subjects were not provided with coaching on how to breathe during the inspiratory loading which resulted in the subject's being able to alter their breathing pattern to minimize respiratory work. For two of them it became a respiratory muscle training session rather than a respiratory muscle fatiguing session evidenced by their increases in MIPS over time. As a result, all remaining subjects were provided with real time visual feedback of the mouth pressure they were generating with each breath so they could hit their target inspiratory pressure with each breath and subjects were also instructed to maintain the inhalation for the entire duration of the inspiratory phase. This resulted in them hitting the target inspiratory pressure and maintaining more of a square wave as pictured in (**Figure 3**). At baseline and following each round of inspiratory loading (baseline, post 1, post 2, and post 3), participants completed measures of lung physiology which include MIPS, resting seated respiratory gas exchange (5 minutes), forced oscillation technique (FOT), exhaled nitric oxide, cardiac output, diffusing capacity of the lungs for carbon monoxide and nitric oxide and basic spirometry. Testing was performed in this order to avoid altering operational lung volume and resting ventilation following the inspiratory loading. Therefore, the order of testing would not interfere or skew the measures of respiratory mechanics, breathing patterns, lung damage, cardiac output, or diffusing capacity.

Prior to the first bout of inspiratory loading and after the last bout of inspiratory loading a lung ultrasound was completed to measure comet tails as an indirect marker of fluid flux across the lungs, i.e., either an index of extravascular fluid or enhanced lymph flow. Each intercostal space from the second to fifth on the right and second to the fourth on the left was scanned in four different positions, parasternal, midclavicular, anterior axillary, and midaxillary. Less than 5 comet tails were considered normal and at least 15 comet tails were considered moderate levels of extravascular lung water. Lung ultrasound was performed before spirometry at both time points.

Figure 2. PowerBreathe Plus, a moderate resistance inspiratory loading device

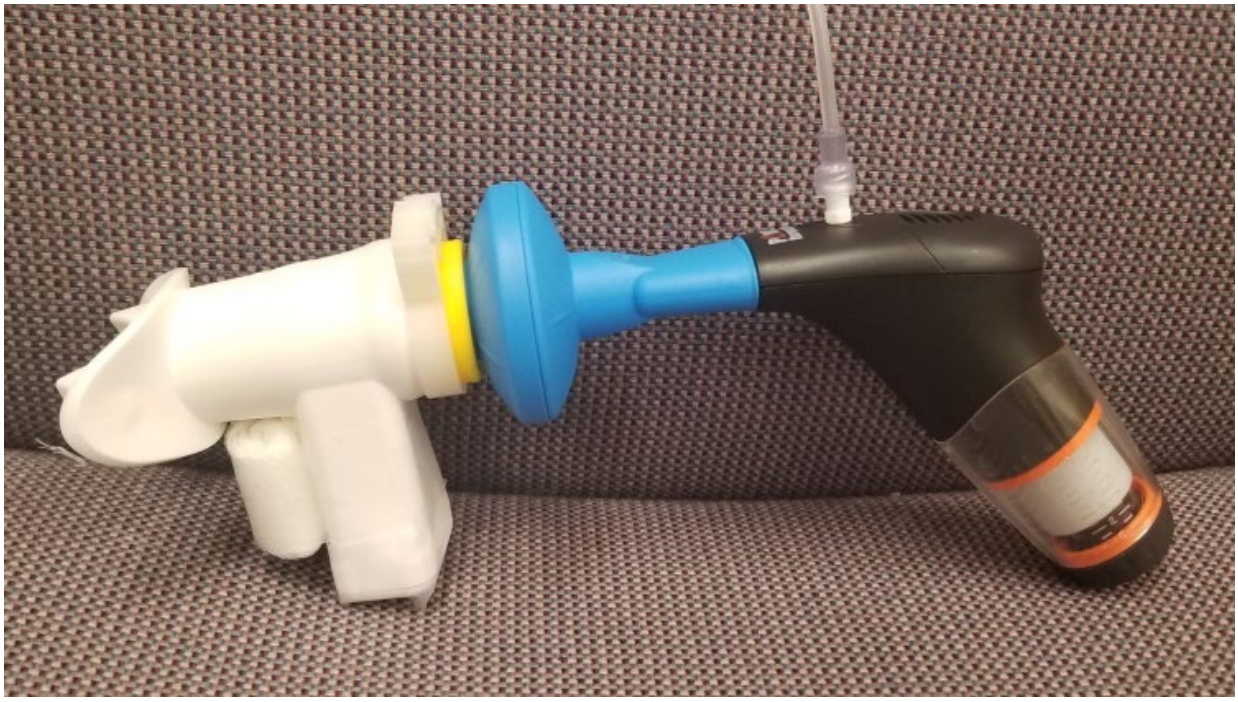
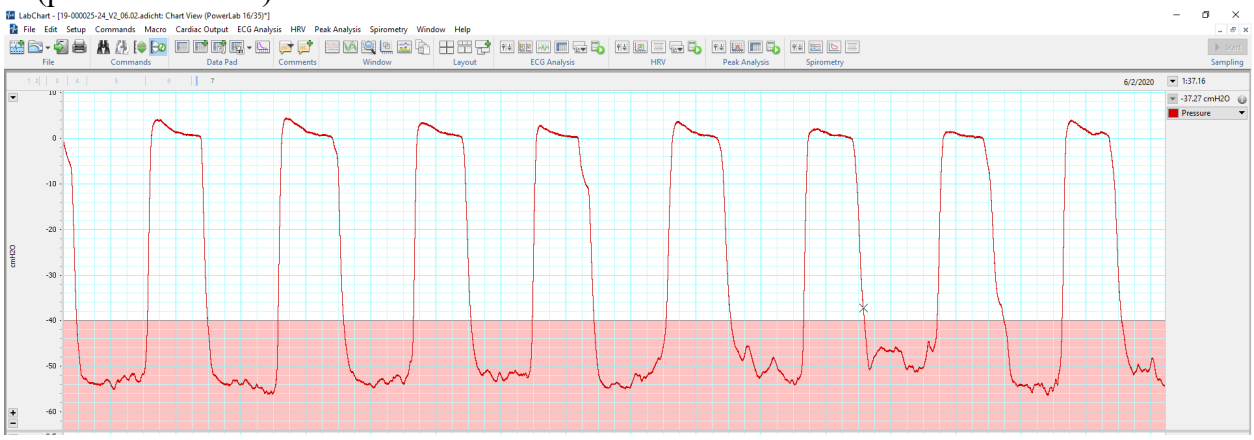


Figure 3. Subjects were coached to hit the pink area and coached to reach a given percent of MIPS (pink shaded area).



3.2 Study Aim 2 Participants

A total of 24 (14 male, 10 female) healthy subjects (mean ± SD age: 29.84 ± 6.44yrs; height: 175.71 ± 11.28cm; weight: 80.73 ± 15.98kg; **Table 2**) with a moderate to high fitness level, completed this study. Subjects performed a positive pressure breathing challenge at either 15 or 20 cmH_2O positive pressure breathing while also experiencing an elastic load, for three 30-minute sessions. The Mayo Clinic Institutional Review Board approved this study for human subjects. The subjects had no known cardiovascular, pulmonary, metabolic, muscular diseases, and/or neural/cognitive disorders. All subjects signed a written informed consent document before testing.

Table 2. Subject demographics for arm 2. Mean ± SD

	ALL	15 cmH_2O	20 cmH_2O
n	24	12	12
Female	10 (42%)	6 (50%)	4 (33%)
Age (years)	29.84 ± 6.44	31.75 ± 6.12	28.17 ± 6.73
Height (cm)	175.71 ± 11.28	175.75 ± 12.69	177.61 ± 7.95
Weight (kg)	80.73 ± 15.98	82.10 ± 17.27	80.49 ± 15.50
BMI (kg/m^2)	26.00 ± 3.85	26.35 ± 3.78	25.41 ± 4.10
FVC (% predicted)	97 ± 13	96 ± 13	98 ± 14
FEV_1 (% predicted)	94 ± 13	93 ± 13	96 ± 14
MIPS (cmH_2O)	107.96 ± 28.29	108.75 ± 31.00	107.09 ± 26.50
MEPS (cmH_2O)	132.65 ± 31.55	138.67 ± 32.58	126.09 ± 30.51
Hemoglobin (g/dL)	14.52 ± 1.24	14.48 ± 1.32	14.57 ± 1.20
Hematocrit (%)	43.32 ± 2.95	43.42 ± 2.97	43.23 ± 3.07

3.2.1 Experimental Design Study Aim 2

The study involved a total of 2 visits, the screening visit, and the test visit (**Figure 3**). Visit 1 consisted of baseline measures and typically lasted 1 hour. Visit 2 took place on the following day and was completed in about 3.5 hours. For the test visit, subjects were randomly assigned to one of two groups (15 or 20 cmH_2O positive pressure breathing). Each subject then performed a series of measures of lung physiology (described below) for baseline reference, followed by elastic abdominal/chest wrapping, with the goal of reducing the vital capacity (FVC) of the subject's lungs by ~20% and performed three separated ventilator sessions for a duration

of 30-minutes each, while repeating the lung physiology measure between each ventilator session.

3.2.2 Visit 1 - Familiarization Study Aim 2

During visit 1, informed consent was obtained, along with measurements of height, weight and vital signs (blood pressure, temperature and heart rate), a baseline ECG, a blood draw to test for anemia (<12g/dl for males, <11g/dl for females), as well as a urine pregnancy test for females of child bearing potential. Visit 1 was used as a screening visit and typically lasted 1 hour. During this visit subjects were randomly assigned to one of two groups (15 or 20 cmH_2O positive pressure breathing), for a total of 12 subjects per group.

3.2.3 Visit 2 – Positive Pressure challenge Study Aim 2

Each subject performed a series of measures of lung physiology (described below) for baseline, followed by elastic abdominal/chest wrapping, to imitate the typical load presented by the upper pressure garments worn by pilots during flight, with the goal of reducing the vital capacity (FVC) of the subject's lungs by ~20%. Subjects were then fitted to a ventilator set to provide either 15 or 20 cmH_2O of pressure during inspiration (based on the subject's randomly assigned group) for a duration of 30-minutes. After each ventilator session, the same series of measures of lung physiology as completed for the baseline measure, were performed again. There were a total three, 30-minute ventilator sessions, with the same series of measures of lung physiology performed after each session.

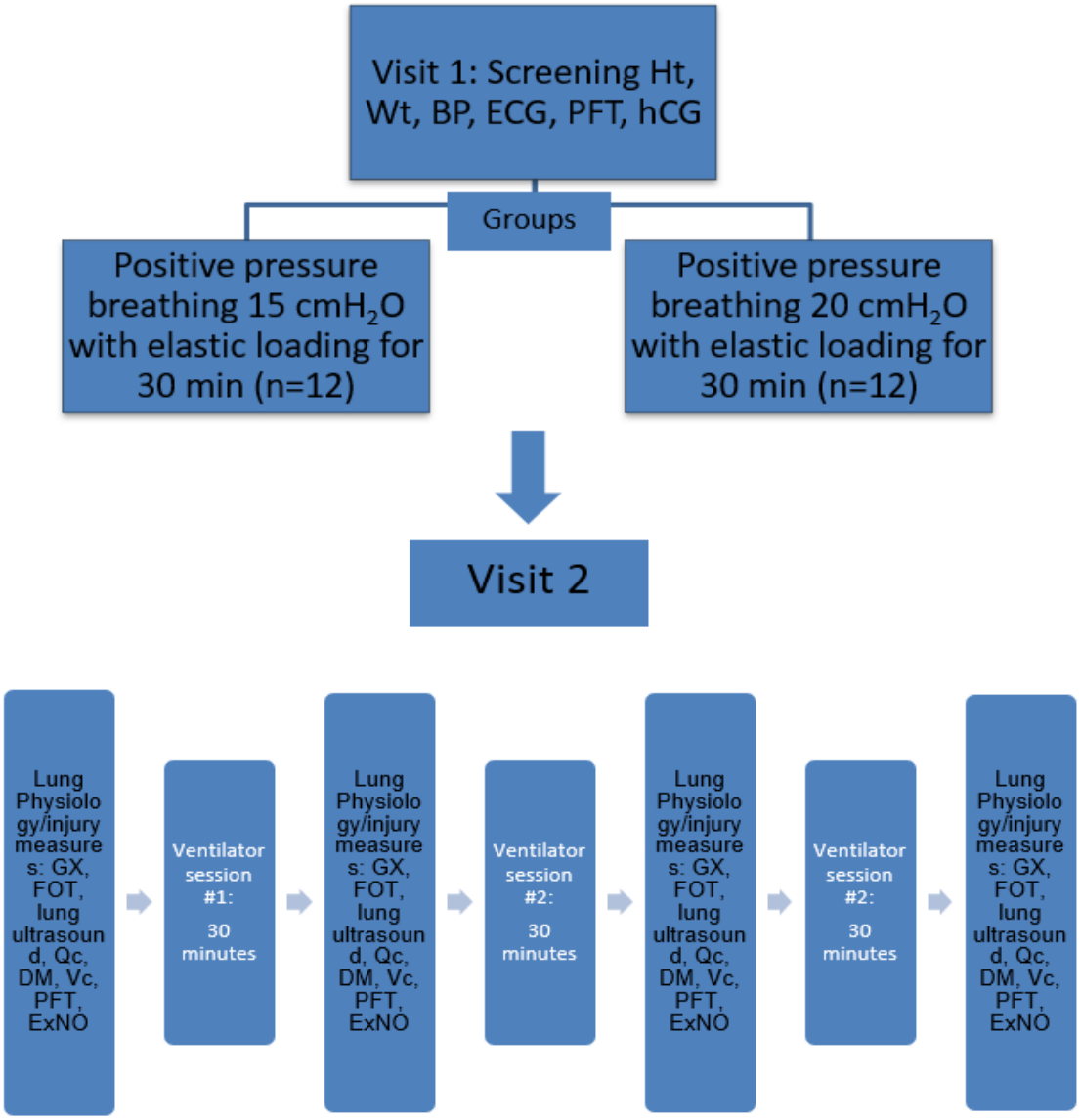


Figure 4. Study Aim 2 Protocol

3.3 Measured and Computed variables

3.3.1 Lung Physiology Measures. At baseline and following each round of ventilator breathing with elastic loading (baseline, post 1, post 2, and post 3), participants completed measures of lung physiology which include resting seated pulmonary gas exchange (5 minutes), forced oscillation technique (FOT), exhaled nitric oxide, cardiac output, diffusing capacity of the lungs for carbon monoxide and nitric oxide and basic spirometry. Testing was performed in this order to avoid altering operational lung volume and resting ventilation following the inspiratory loading. Therefore, the order of testing would not interfere or skew the measures of respiratory mechanics, breathing patterns, lung damage, cardiac output, or diffusing capacity.

3.3.1.1 Gas Exchange. Oxygen consumption (VO_2), exhaled carbon dioxide (VCO_2), tidal volume (Vt), respiratory rate (RR), minute ventilation (VE), respiratory exchange ratio (RER), ventilatory equivalent for carbon dioxide (VE/VCO_2), End tidal carbon dioxide ($ETCO_2$), heart rate (HR), and oxygen saturation (SpO_2) were measured immediately after each bout of respiratory loading. Subjects were in a seated position and instructed to breath normally on a mouthpiece attached to a pneumotach. The Ultima CPX metabolic cart (MGC, St. Paul, MN) was used to measure gas exchange variables. All gas exchange variables were measured at baseline, post 1, post 2, and post 3 during visit 2. HR and SpO_2 were measured using a pulse oxygenation sensor placed on the forehead (Massimo, CA, USA).

3.3.1.2 FOT – Resistance and Reactance. Resistance and reactance of peripheral and central airways was measured using a forced oscillation technique (FOT) (Resmon Pro Full, MGC Diagnostics Corp., St. Paul, MN). Subjects were seated in an upright position and instructed to

breath normally on the FOT device. Three different frequencies, 5, 11, and 19 Hz were used to measure both central and peripheral obstruction.

3.3.1.3 Exhaled nitric oxide. ExNO was measured using the handheld FeNObreath (Medical Graphics Corporation, St. Paul, MN). In a seated position, subjects were instructed to take a deep inhale off the machine and exhale slowly and completely on the handheld device. Normal ExNO was <25 ppb.

3.3.1.4 Lung Diffusion, Quantification of alveolar capillary membrane conductance, pulmonary capillary blood volume and Cardiac Output. Lung diffusing capacity for carbon monoxide (DLCO) and nitric oxide (DLNO) as well as pulmonary blood flow (Qc) were measured simultaneously with the subjects in a seated position using a rebreathing technique with a 5-liter anaesthesia bag containing 0.7% acetylene, 9% helium, 0.3% carbon monoxide (C18O), 40 PPM NO (diluted immediately before the test in the bag from an 800 PPM gas mixture) and 35% O2, at a respiratory rate of 32 breaths/minute as described previously [16-18]. For all measurements, the bag volume matched the individual's tidal volume plus 500mL, with a minimum bag volume of 1000mL, to ensure the bag did not collapse during inhalation, but also did not cause an unnecessary excess of gas in the bag during the manoeuvre. At the end of a normal expiration (functional residual capacity), the subjects were switched into the rebreathe bag and instructed to nearly empty the bag with each breath for 8-10 consecutive breaths. The manoeuvre was performed in triplicate at each time point.

The rate of disappearance of acetylene from the exhaled gas mixture during rebreathing is used to assess pulmonary blood flow. Acetylene is highly soluble in the blood, therefore, the rate of disappearance of acetylene is limited primarily by the rate at which a new volume of blood is

transported through the lungs. Because the blood passing through the lungs is about 98-99% of the cardiac output, this measure of the disappearance of acetylene provides a reliable measure of cardiac output and has previously been validated in our laboratory using direct Fick during exercise [19, 20].

3.3.1.5 Spirometry. Basic spirometry included measurement of slow forced vital capacity (SVC), forced vital capacity (FVC), minute ventilation (MVV), inspiratory capacity (IC), forced expiratory volume in one second (FEV_1), forced expiratory flow between 25 and 75% of FVC (FEF_{25-75}), maximum forced expiratory flow (FEFmax), forced inspiratory vital capacity (FIVC), 50% of forced expiratory flow to 50% of forced inspiratory flow ratio (FEF_{50}/FIF_{50}), and maximum forced inspiratory flow (FIFmax). In a seated position subjects were instructed to breathe normally on a mouthpiece attached to a pneumotach and the Ultima CPX metabolic cart. Subjects were instructed and coached through each breathing maneuver. To measure SVC subjects took a maximal inhale on the mouthpiece and slowly and completely exhaled on the mouthpiece. Following the measure of SVC, subjects were asked to take a maximal inhale and quickly and forcefully exhale holding the exhale for at least 6 seconds to measure FVC, FEV_1, FEF_{25-75}, FEFmax, FIVC, FIF max and FEF_{50}/FIF_{50}. MVV was measured by instructing subjects to take a deep and fast breaths moving as much volume as possible over 30 seconds. All spirometry maneuvers were repeated at least three times.

3.3.1.6 Comet Tails. The assessment of lung atelectasis and fluid flux were measured from the appearance of comet tails (hyperechoic reflections) within the right (second to fifth) and left (second to fourth) intercostal spaces in four different positions (parasternal, midclavicular, anterior axillary and mid-axillary) through the use of a lung ultrasound (Philips Lumify). The presence of

comet tails was assessed at baseline and again after the third ventilator session. Lung ultrasound was performed before spirometry at both time points.

3.3.2 Inspiratory fatigue. MIPS, the change in MIPS, and total time under inspiratory load were measured during visit 2. MIPS was measured at baseline, post 1, post 2, and post 3. Post 1, post 2, and post 3, occurred after the first, second, and third bout of the inspiratory challenge, respectively (**Figure 1**). Change in MIPS was calculated by subtracting the MIPS measured following each round of inspiratory loading from baseline measured MIPS. The total time under the inspiratory load the number of minutes subjects were able to tolerate breathing against either 40 or 60% of their MIPS. Additionally, total time with inspiratory threshold loading, percent of target inspiratory threshold loading, and percent change in MIPS were calculated. The total duration spent breathing against an inspiratory load was calculated as the summation of the total time in minutes each individual spent breathing against the inspiratory load for each round. The percent of target was the percentage of the individuals 40 or 60% of MIPS target workload that each individual was actually able to tolerate and sustain over the entire inhalation during inspiratory loading. As a measure of energy expenditure or respiratory muscle effort the pressure-time product (PTP) was used as it represents the time under tension during inspiratory loading over measured mouth pressure. Respiratory muscle effort was expressed as the pressure-time product (PTP) which was quantified as the product of the average inspiratory pressure (Piavg) and the duration of inspiration (Ti): PTP = Piavg × Ti or the average expiratory pressure (Peavg) and the duration of expiration (Ti): PTP = Peavg × Te for inspiratory and expiratory phases respectively. This was calculated from the mouth pressure signal measured during inspiratory loading. This was used as a measure of energy expenditure or respiratory muscle work as it represents the time under tension during inspiratory loading. Respiratory muscle effort was summed over all breaths for each round of

inspiratory loading and as well summing the total for each round to get a total respiratory muscle effort or work over the entire visit.

3.3.3 Elastic Load and Ventilator Sessions. Elastic wrapping, using two 4" by 3.2 yard elastic wraps (SurePress), began by asking the subject to exhale out to residual volume (RV) while the subject's abdomen and chest were wrapped to achieve a ~20% reduction in FVC. Repeated FVC measure confirmed reduction, then the subject was seated in an upright position and placed on the ventilator. The ventilator was set in the spontaneous/timed mode to provide either 15 or 20 cmH_2O pressure (depending on group) at an respiratory rate of 15 breaths per minute, with 5 cmH_20 expiratory positive pressure and the inspiratory time of two seconds one breath made up 50% of the total breath time (Ti/TTot) for 30 minutes. Subjects were instructed to try their best to breathe normally, that if wanted they could let the ventilator breathe for them and there were shown how to let the ventilator initiate a breath. The elastic wrapping and 30-minute ventilator sessions were repeated for a total of three sessions, with the previously described lung physiology measurements being taken before and after each session.

3.3.4 Assessment of Breathing Desynchrony with the Ventilator. The pressure-time product (PTP) which was quantified as the product of the average inspiratory pressure (Piavg) and the duration of inspiration (Ti): PTP = Piavg × Ti or the average expiratory pressure (Peavg) and the duration of expiration (Ti): PTP = Peavg × Te for inspiratory and expiratory phases respectively. This was calculated from the mouth pressure signal measured during each round of ventilator breathing sampled from the top sample port located above the nose on non-vented full-face mask. This was used as means to understand 1) respiratory muscle effort and 2) assess and identify individuals who were fighting or struggling to breathe with the ventilator. For quantification of respiratory muscle effort, the PTP was summed over all breaths for each round of ventilator

breathing and as well summing the total for each round to get a total respiratory muscle effort over the entire visit. To assess struggling with the ventilator the difference between each individual's average breath to breath PTP and the mean average breath to breath PTP was calculated where those who demonstrated a negative difference where labelled as struggling. The variability in the breath to breath PTP within each individual across each round and across the entire visit was also used as a feature of fighting the ventilator. While breathing on the ventilator Vt, Fb, VE, inspiratory time over total breath time (Ti/TTot) and percentage of breaths that were patient triggered were recorded and averaged every minute for each round of ventilator breathing.

3.4 STATISTICAL ANALYSIS

3.4.1 Study Aim 1. Data for all subjects were averaged and inspected for patterns between groups and overtime. These data were summarized using proportions, means, standard deviations, medians, interquartile ranges, and linear trends over time. Mixed factorial ANOVAs were run when appropriate to examine the effect of repeated inspiratory loading bouts on various measures of lung physiology between groups.

3.4.2 Study Aim 2. Separate two (independent factor: 15cmH_2O, 20cmH_2O) × four (repeated factor: baseline, post 1, post 2, post 3) mixed factorial ANOVAs and paired samples t-test were used to determine if there were significant differences for all the lung physiology measures.

An alpha level of $p \leq 0.05$ was considered statistically significant for all comparisons. All statistical analyses were performed with Statistical Package for Social Sciences software (v.27 IBM SPSS Inc., Chicago, IL, USA) and GraphPad Prism (v.9, GraphPad Software, San Diego, CA).

4.0 RESULTS AND DISCUSSION

4.1 Study Aim 1

4.1.1 Inspiratory fatigue

MIPS significantly decreased across time, however, there were no significant differences between the 40% vs 60% load groups (p=0.005 & p=0.72, respectively). MIPS decreased from 105.7±27.9, 100.0±27.8, 95.4±27, 94.9±26.9 cm H_2O at baseline to post 1, post 2, and post 3 for subjects in the 40% MIPS group, respectively. Similarly, MIPS decreased from 105.3±31.3, 99.1±32.6, 99.1±27.6, and 95.3±29.8 cmH_2O at baseline to post 1, post 2, and post 3 for subjects in the 60% MIPS group, respectively (**Figure 5**). The duration individuals were able sustain breathing under the inspiratory load significantly decreased with repeated loading and between groups. Subjects in the 40% MIPS group had a decrease in time from 24.8±7.2, 24.1±6.3, 22.6±7.0 minutes during the first, second, and third, inspiratory challenge, respectively. Similarly, subjects in the 60% of MIPS group had a decrease in time from 22.2±6.8, 20.5±8.5, 16.1±8.1 minutes from the first, second, and third inspiratory challenge, respectively (**Figure 6**, p=0.048).

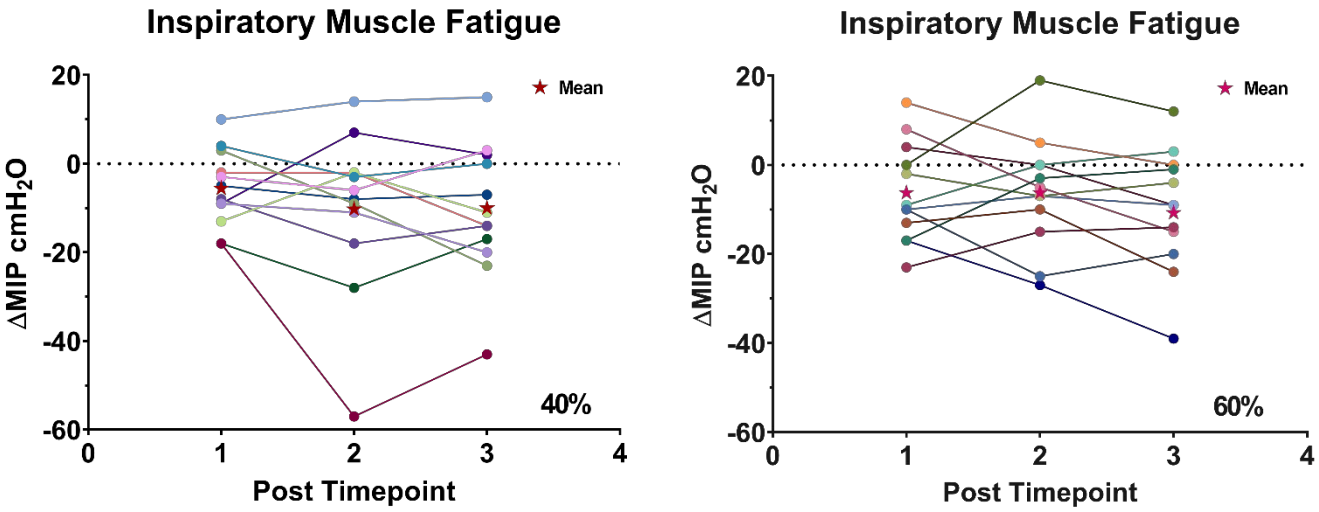


Figure 5. The change in MIPS from baseline across time (post 1, post 2 and post 3) within subject separated by group with 40% loading left panel and 60% loading right panel. Where the dashed line designates zero change, and the star identifies the mean change for each group.

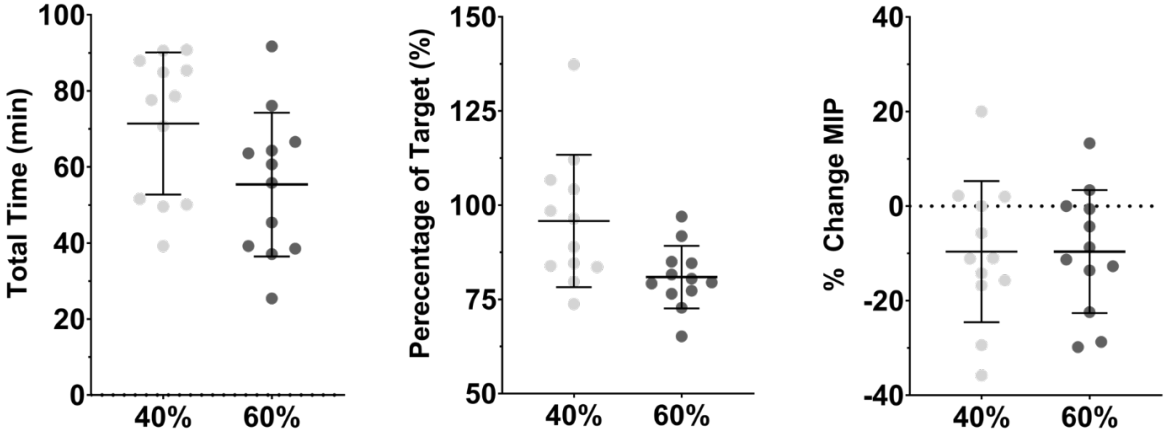


Figure 6. The total time spent breathing against either 40% or 60% of MIPS, percent of target MIPS achieved during repeated inspiratory loading, and the percent change in MIPS from baseline to post 3.

There was a decrease in the total time subjects were able to tolerate the inspiratory threshold with each round of loading for both groups. Subjects breathing against 60% of MIPS had a significantly greater decrease in time compared to subjects breathing against 40% of MIPS. Subjects in the 40% MIPS group had a decrease in total time of 0.45 and 1.3 minutes from post 1 to 2 and post 2 to 3, respectively. Subjects in the 60% MIPS group had a decrease in total time of 3.4 and 2.2 minutes from post 1 to 2 and post 2 to 3, respectively. Therefore, an inspiratory load of 40% of MIPS was better tolerated by subjects compared to an inspiratory load of 60% of MIPS. The percentage of target that the participants in each group were able to breathe against for a prolonged period was lower for the 60% group, such that they were often breathing against closer to 50% MIPS rather than 60%. The repeated inspiratory loading caused subjects to demonstrate respiratory muscle fatigue with a decrease in MIPS they could generate over time, relative to baseline. There was no difference in the degree of change in MIPS between groups from baseline to post 3.

4.1.2 Pressure time product (PTP)

Subjects in the 40% MIPS group were able to withstand a longer total duration of inspiratory loaded breathing compared to subjects in the 60% MIPS group (724 vs 607 seconds). PTP accounts for respiratory muscle effort or work during the phases of respiration, subjects in the 40% MIPS group were able to breath against a lower threshold for a longer duration compared to the 60% MIPS group that were breathing in against a higher inspiratory load, but for a shorter duration. The subjects in the 40% MIPS and the 60% MIPS had a total PTP of 79 and 101 $cmH_2O \cdot s$, respectively. Subjects in the 60% MIPS group had a higher PTP or performed more respiratory muscle but sustained it for a slightly lower duration compared to subjects in the 40% MIPS (**Figure**

7). Overall, 42, 54, 67, and 54% of subjects were below the average for the total time, inspiratory PTP, expiratory PTP, and total PTP, respectively.

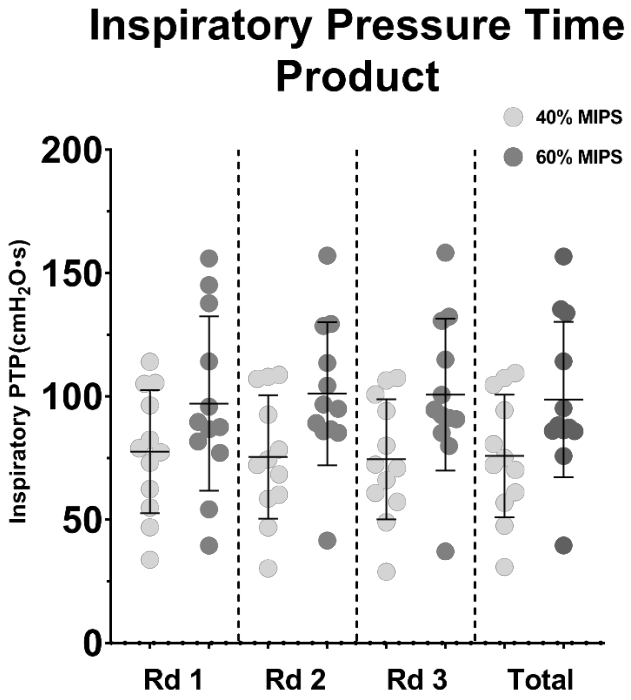


Figure 7. The total average PTP

The total inspiratory PTP was significantly and negatively correlated with the change in DLCO/Q. As the inspiratory PTP increased, the change in DLCO/Q decreased ($r = -0.411$, $p = 0.046$, **Figure 8**). The negative relationship between the change in DLCO/Q and total inspiratory PTP indicates that higher levels of respiratory muscles effort or work during inspiration can negatively impacts DLCO/Q suggesting it could start to affect alveolar-capillary recruitment. Inspiratory PTP during the first round of inspiratory loading was significantly and positively correlated with Vc ($r = 0.414$, $p = 0.049$). The first round of inspiratory loading caused an increase in the amount of blood circulating within the pulmonary vasculature. This was an acute increase and was not significantly correlated with total PTP or with inspiratory PTP for rounds 2 and 3 of inspiratory loading.

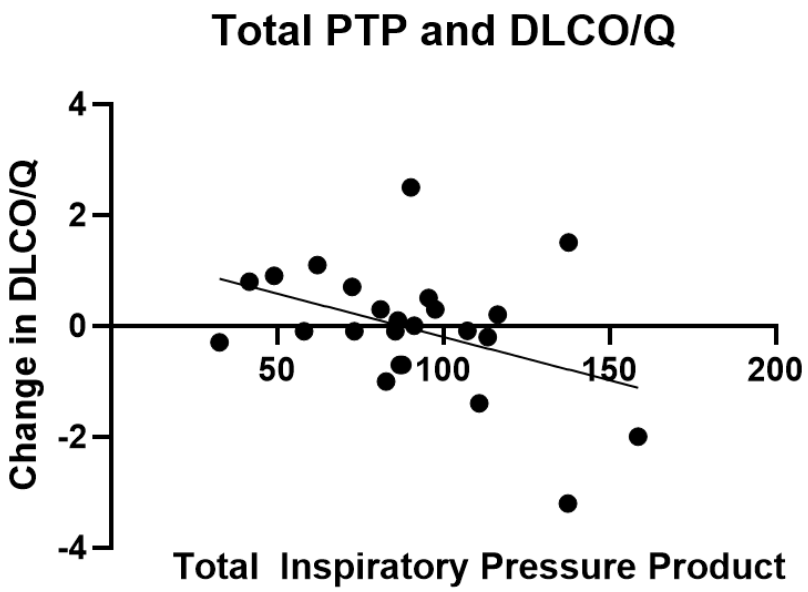


Figure 8. Correlation between PTP and DLCO/Q

4.1.3 Breathing patterns and Gas exchange

There was a significant decrease in measures of resting VCO_2, VT, VE with repeated inspiratory loading, but there was no difference in the change in VCO_2, VT, or VE between groups. VCO_2 decreased from 267±92, 230±73, 205±54, and 192±60 ml/min at baseline, post 1, post 2, and post 3, respectively (p=0.00, **Figure 9A**). Similarly, VT decreased from 708±259, 617±257, 558±206, and 544±201 ml, at baseline, post 1, post 2, and post 3, respectively (**Figure 9B**). VE decreased from 9.8±2.9, 9.7±3.0, 8.6±2.4, and 8.5±3.3 L from baseline to post 1, post 2, and post 3, respectively (p=0.03, **Figure 9C**). There was a significant increase in respiratory rate (RR) with repeated inspiratory loading across time and between groups. Subjects in the 40% of MIP group had an increase in RR from baseline to post 1 and again from post 2 to post 3 15±4, 18±6, 17±6, and 19±7 bpm, respectively (p=0.02, **Figure 9D**). Whereas, subjects in the 60% MIPS group remained the consistent from baseline to post 1, increased from post 1 to post 2 and decreased from post 2 to post 3, 14±3, 14±2, 16±3, and 15±2 bpm, respectively (p=0.02, **Figure 9E**).

Furthermore, there was a significant increase in VE/VCO_2 with repeated inspiratory loading at 37.7±5.5, 43.3±6.8, 44.4±5.5, and 46.9±6.7 from baseline, post 1, post 2, and post 3, respectively (p=0.00). $ETCO_2$ decreased from 34.2±5.4, 31.7±5.5, 31.8±5.0, and 31.1±4.7 mmHg, at baseline, post 1, post 2, and post 3, respectively (**Figure 10**, p=0.01).

Figure. 9A-E

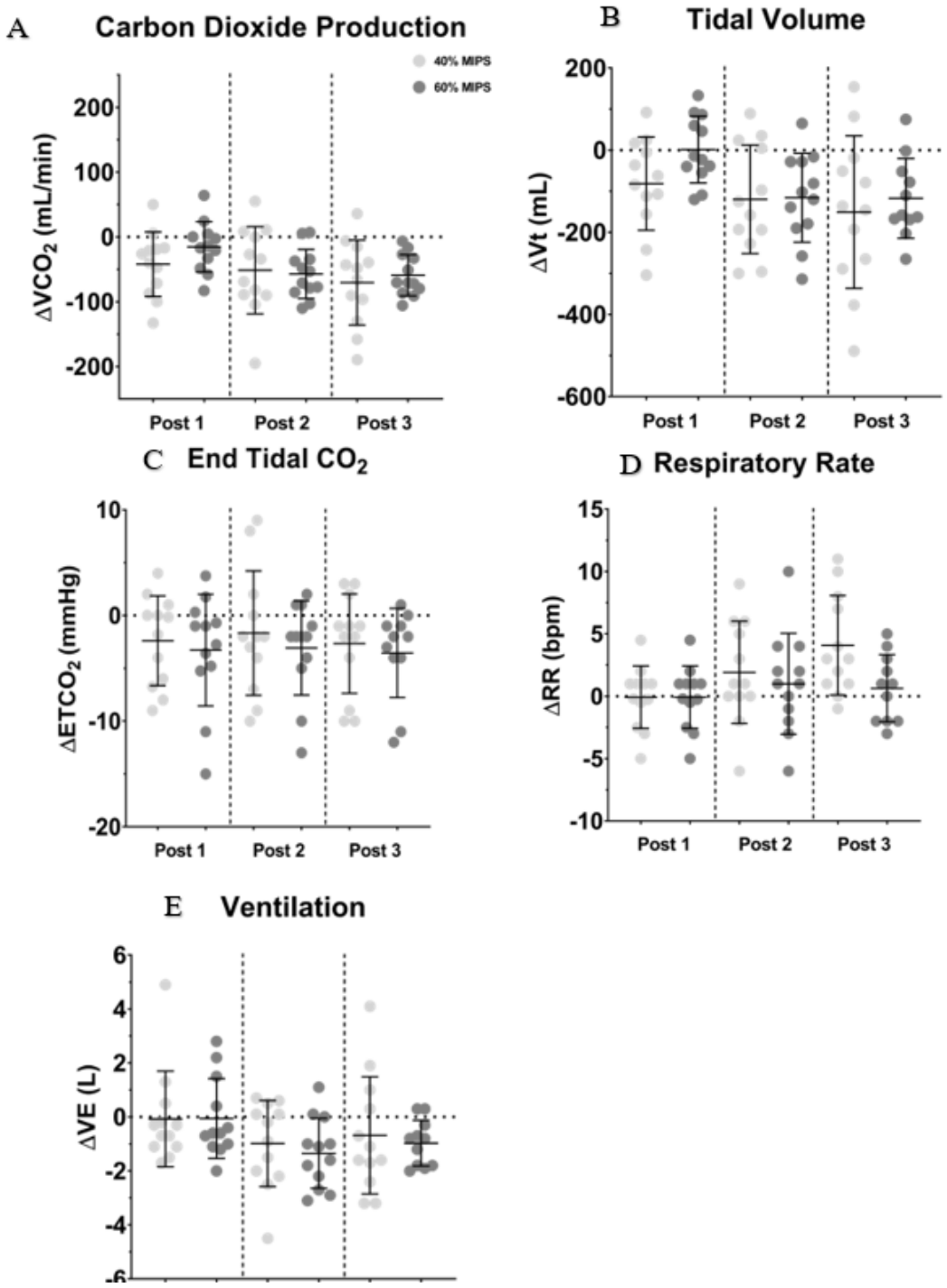


Figure 9A. Change in VCO2 across time, **Figure 9B**. Change in Tidal Volume Across Time. **Figure 9C.** Change in VE across time, **Figure 9D**. Change in RR across time, **figure 9E.** Change in ETCO2 across time

4.1.4 Lung Mechanics: Reactance, Resistance, and Spirometry

There were nonsignificant changes in inspiratory, expiratory, and total reactance and resistance at the three different frequencies between groups and across time with repeated inspiratory loading ($p > 0.05$). There was a small but significant decrease in SVC, FVC, and FEV_1 with repeated inspiratory loading, however there were nonsignificant differences between subjects in both the 40 and 60% MIPS groups (**Table 3, Figure 10A-D**). MVV Vt (L) decreased with repeated inspiratory loading. Repeated inspiratory loading caused subjects to take more shallow breaths in the time immediately post loading. There were no significant changes in the other spirometry measures examined ($p>0.05$). Although, there were subtle declines in SVC, FVC, and FEV_1 with repeated inspiratory loading as noted there were no changes in resistance or reactance measured with FOT. Thus, the decline in SVC, FVC, FEV1 may be a result of the respiratory muscle fatigue and not indicative of true pathological changes in lung physiology. Furthermore, there were no significant differences in ExNO with repeated inspiratory loading or between groups ($p> 0.05$, Figure 8). The nonsignificant changes in ExNO suggest that repeated inspiratory loading did not induce inflammation of the airways (**Figure 10D**).

Table 3. Change in spirometry across time

	Baseline	Post 1	Post 2	Post 3
SVC (L) *effect of time (p=0.001)				
All	4.57±1.10	4.53±1.11	4.43±1.13	4.43±1.09
40%	4.60±1.26	4.56±1.29	4.47±1.33	4.49±1.24
60%	4.54±0.97	4.51±0.95	4.40±0.96	4.38±0.98
FVC (L) *effect of time (p=0.001)				
All	4.71±1.11	4.61±1.12	4.55±1.11	4.56±1.09
40%	4.78±1.32	4.67±1.34	4.57±1.32	4.60±1.32
60%	4.64±0.92	4.56±0.91	4.53±0.91	4.53±0.91
FEV_1 (L) * effect of time (0.002)				
All	3.99±0.79	3.94±0.84	3.86±0.85	3.87±0.84
40%	4.04±0.86	4.01±0.89	3.89±0.89	3.92±0.84
60%	3.93±0.75	3.88±0.82	3.83±0.85	3.82±0.88
FEF25-75 (L/sec)				
All	4.27+0.87	4.27±1.03	4.18±1.09	4.16±1.06
40%	4.38±0.98	4.46±1.02	4.27±1.02	4.35±1.06
60%	4.17±0.77	4.08±1.05	4.09±1.20	3.97±1.20
MVV (L)				
All	140±34	137±36	134±34	134±39
40%	142±31	141±41	135±34	138±42
60%	138±37	133±30	134±34	131±37
MVV Vt (L) * effect of time (p=0.04)				
All	2.48±1.25	2.52±1.16	2.39±1.22	2.43±1.36
40%	2.67±1.27	2.64±1.18	2.37±1.27	2.47±1.57
60%	2.50±1.27	2.41±1.19	2.40±1.22	2.40±1.18

Figure 10 A-D

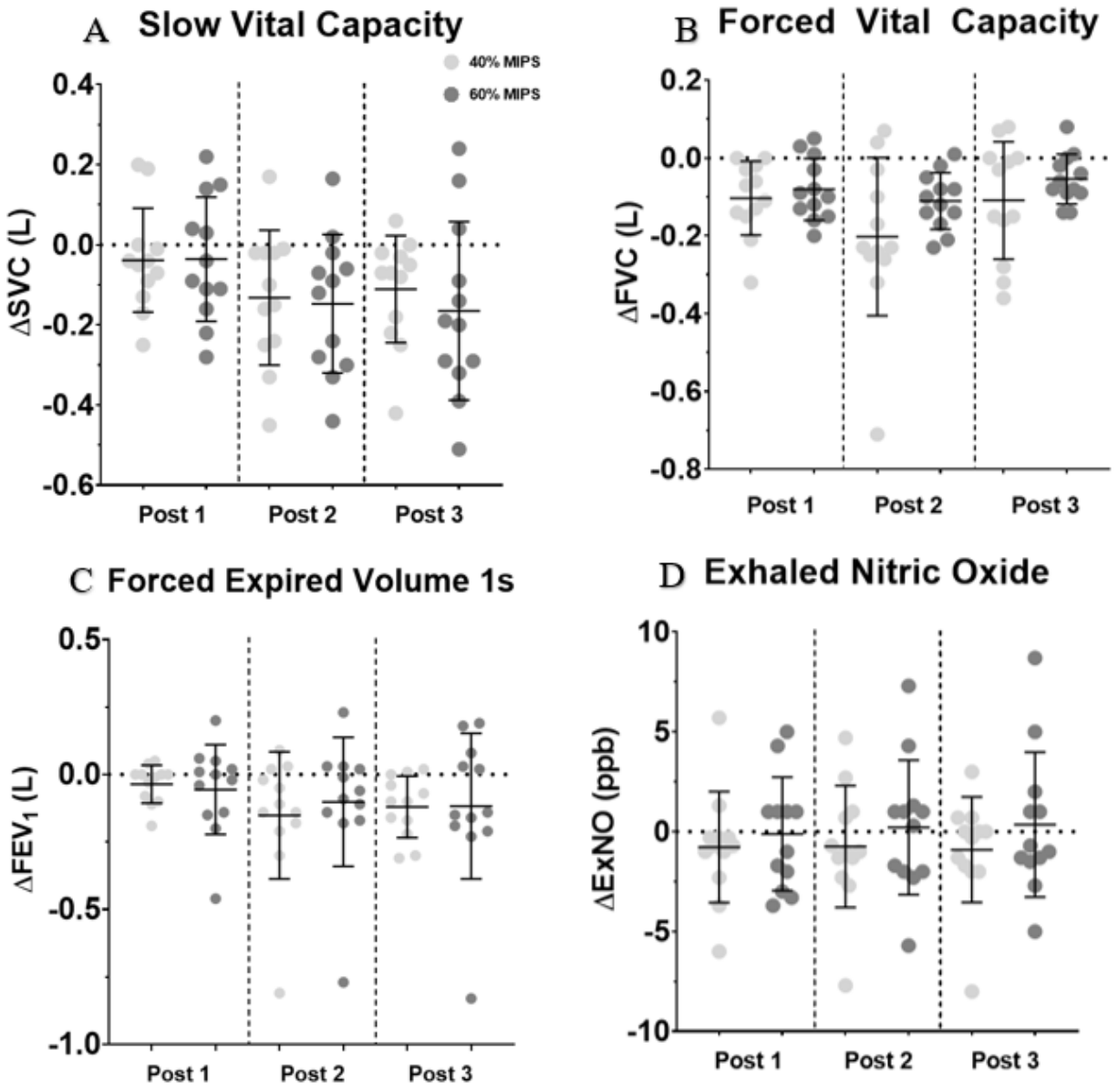


Figure 10A. Change in SVC across Time, **Figure 10B.** Change in FVC across time, **Figure 10C.** Change in FEV1 across time, **Figure 10D.** Change in ExNO across time

4.1.5 Hemodynamics and Pulmonary Vasculature

The alveolar capillary membrane is thin enough to permit gas exchange and is highly vulnerable to mechanical stress. Inspiratory loaded breathing provides an additional stressor to the lungs and requires a greater force of contraction to overcome the additional resistance leading to large negative swings in intrathoracic pressure which can result in damage to the alveolar capillary membrane.[4] Although previous research has shown that inspiratory loaded breathing can lead to decrements in lung function, we did not observe significant changes in alveolar – capillary membrane conductance or the more global measure of lung transfer factors for CO or NO. Since this measure is sensitive to changes in pulmonary blood flow or cardiac output and alveolar volume, they are expressed these values are expressed relative to these measures as well as alone. There were non-significant changes in the lung gas transfer across the alveolar capillary membrane (DLCO) or the surface area for gas exchange in the lungs (DM), cardiac output (Q), alveolar capillary recruitment (DLCO/Q), or functional unit of diffusion (DM/Vc) with repeated inspiratory loading and between groups ($p>0.05$, Figures 11A-16D).

Figures 11A-E

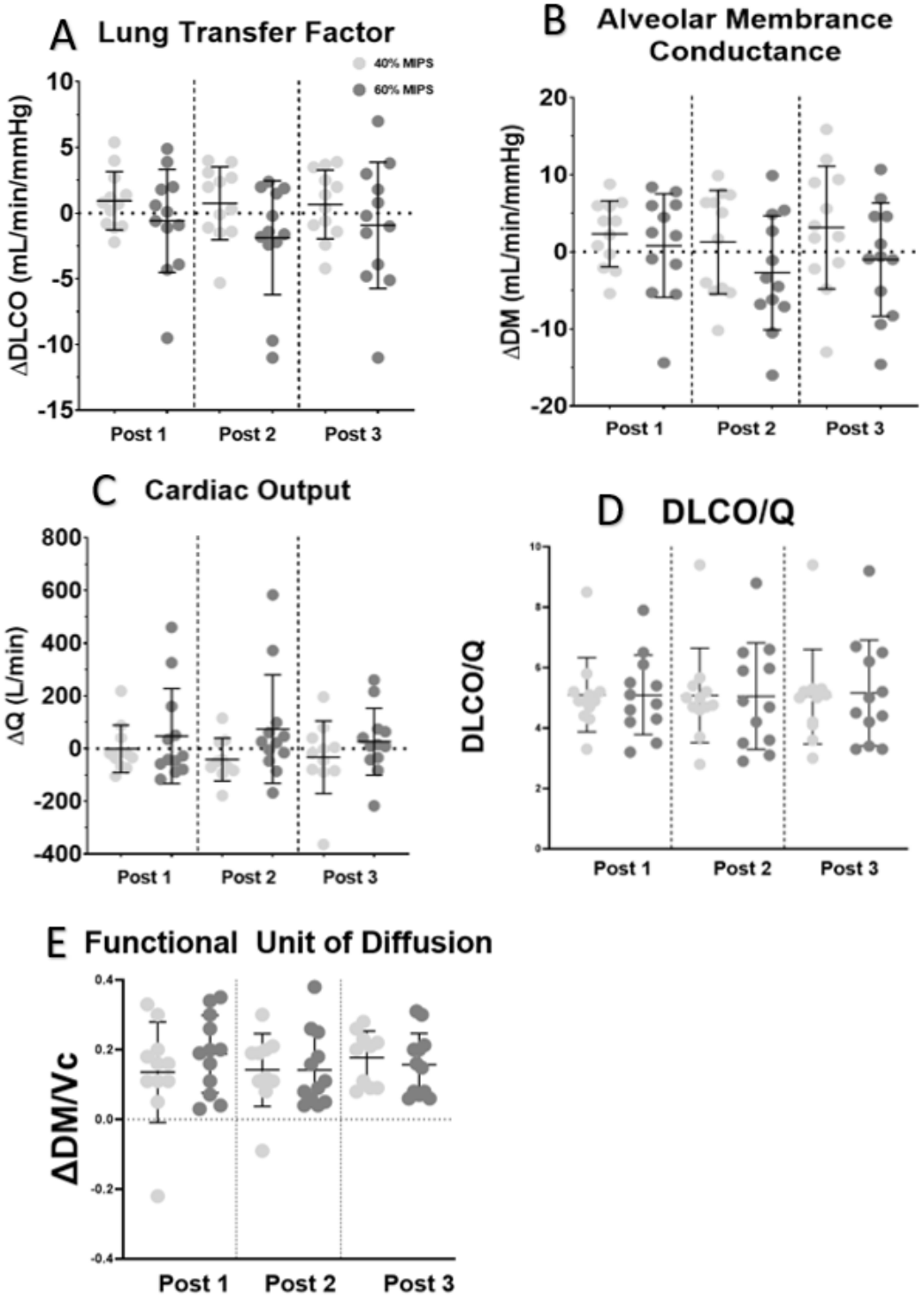


Figure 11A. Change in Lung Transfer Factor, **Figure 11B.** Change in Alveolar Membrane Conductance, **Figure 11C.** Change in Q across time, **Figure 11D.** Change in Alveolar Capillary Recruitment, **Figure 11E.** Change in evaluation of the Functional Unit of Diffusion

4.1.6 Outliers

All data were screened for patterns and outliers. Although, there was variability within the measures of lung function and lung physiology, only one subject was considered a significant outlier due to the more severe changes in lung function and physiology. The subject was male, 25 years old, had a BMI of 27 kg/m^2, and was randomized to the 40% MIPS group. The subject spent a total of 71 minutes of threshold loaded breathing and had a total decline in MIPS of 11% from that baseline to post 3. The subject had an increase in VO_2 (ΔPost 3: 101 mL/min/kg), Q (ΔPost 3: 1.5 L/min), Vc (ΔPost 3: 196 mL), comet tails (ΔPost 3: -1), and lung resistance at all frequencies (ΔPost3 R5: 0.82 Hz; R11: 0.65 Hz; R19: 0.50 Hz). The subject had decreases in VT (ΔPost 3: 82 mL), SVC (ΔPost3: -220 mL), FVC (ΔPost3: -170mL), FEV_1 (ΔPost3: -100mL), MVV VT (ΔPost3: -580 mL), DLCO (ΔPost 3: -2.4 mL/min/mmHg), DM (ΔPost 3: -13 mL/min/mmHg), reactance at all levels (ΔPost3 X5: 0.82 Hz; X11:-0.22 Hz; X19: -0.31 Hz) and exhaled NO (ΔPost3: -8 ppb). However, the subject reported no symptoms 24 hours after the repeated inspiratory loading. The more dramatic changes observed in this subject may be related to the individual's ability to generate a very high MIP at 126 cmH_20, which was 105% of predicted, which was the highest in the 40% group, but half of the subjects (n=6) in the 60% group were breathing against a higher inspiratory load during their sessions. Although, this subject had a higher than predicted MIPS, the total PTP or respiratory work performed by this subject over the three rounds was 83 cmH_2O·s which was similar to the average for the other subjects in the 40% MIPS group at 79 ± 25 cmH_2O·s and below than the average for subjects in the 60% MIPS group at 101 ± 32 cmH_2O·s. Thus, despite the higher baseline MIP, the subject had similar total load exposure to most of the other subjects. It is unclear if this subject may be an example of a very small subset of individuals with some higher level of susceptibility of lung changes with loaded breathing. The fact that this subject was consistently the outlier across the three time points for

multiple parameters suggests that this would only be bad data if the baseline measure was off, but these measurements do not appear abnormal or off.

4.2 Study Aim 2

4.2.1 Positive pressure breathing in the presence of an elastic load

The elastic wrapping/load for the ventilator sessions caused a significant ($p = 0.001$) average decrease in FVC (3.70 ± 0.91 L) compared to the average of the four (baseline, post 1, post 2, post 3) unwrapped FVC measures (4.77 ± 1.00 L). For an average decrease of 22.41% (**Table 4**) in FVC during the ventilator sessions due to the elastic wrapping/load. There was a significant average increase in Vt ($p = 0.001$) and Fb ($p = 0.001$) during the ventilator sessions (1014.78 ± 395.29 ml; 20.04 ± 4.45 bpm) compared to the average of the four unwrapped pulmonary gas exchange measurements Vt (713.12 ± 286.56 ml) and Fb (15.17 ± 3.33 bpm). Leading to an average increase of 52% in Vt and 36% in Fb during the elastic load ventilator sessions. The average PtP for each round, along with the total PtP is shown in **Table 5**. There was a noticeable difference in the respiratory pattern within subjects who did not struggle (n = 10) or resist the ventilator assistance (**Figure 12(A)**), in which the example shows the subject taking smooth, long duration inhales and exhales. Whereas those subjects that did struggle (n = 8) and resist the ventilator assistance (**Figure 12(B)**) resulted in a short, choppy respiration pattern. The subjects that struggled during the ventilator sessions showed a significant higher Fb (24.90 ± 4.76 bpm; $p = 0.029$), V_E (30196.00 ± 10207.70 ml; $p = 0.025$) and number of breaths taken (727.88 ± 141.94; $p = 0.003$), with a significantly lower total inspiratory (12.90 ± 2.62; $p = 0.001$) and total (19.06 ± 3.68; $p = 0.001$) PtP, compared to the subject who did not struggle during the ventilator

sessions (Fb 17.19 ± 2.31 bpm; V_E 14477.60 ± 6546.60 ml; #breaths 509.87 ± 46.11; total inspiratory PtP 20.33 ± 2.80; and total PtP 27.47 ± 2.66).

Table 4. Individual subject's FVC percent (%) reduction due to elastic wrapping.

Subject	Round 1 % FVC Reduced	Round 2 % FVC Reduced	Round 3 % FVC Reduced	Total % FVC Reduced
1	13.03	8.50	1.18	7.57
2	29.18	36.01	30.60	31.93
3	14.26	22.17	28.97	21.80
4	26.15	18.35	18.45	20.98
5	11.99	16.83	18.50	15.77
6	26.27	33.71	42.36	34.12
7	3.94	16.12	32.30	17.46
8	29.64	25.50	24.38	26.51
9	14.65	27.14	33.61	25.13
10	30.00	44.40	44.56	39.65
11	35.26	29.92	40.16	35.11
12	21.23	24.12	17.22	20.85
13	22.05	28.95	16.70	22.57
14	18.14	19.41	20.55	19.37
15	22.32	22.62	21.68	22.21
16	21.37	18.10	18.77	19.41
17	15.76	16.11	14.66	15.51
18	23.16	17.76	23.06	21.33
19	24.15	14.98	11.55	16.89
20	17.66	21.57	20.75	19.99
21	18.25	11.53	18.00	15.93
22	28.87	17.86	23.48	23.41
23	19.31	21.45	21.01	20.59
24	26.56	21.84	22.95	23.78
Average	**21.38**	**22.29**	**23.56**	**22.41**
SD	**7.12**	**8.06**	**9.94**	**7.09**

Table 5. Mean ± SD for the total # of breaths, inspiratory, expiratory and total PtP for each of the three positive pressure breathing with elastic loading sessions.

	# of breaths	Inspiratory PtP	Expiratory PtP	Total PtP
Round 1				
All	619.11 ± 149.74	16.78 ± 4.78	6.49 ± 1.79	23.27 ±5.39
15 cmH_2O	555.13 ± 156.57	16.29 ± 4.77	7.48 ± 1.99	23.77 ± 5.15
20 cmH_2O	670.30 ± 126.49	17.17 ± 5.02	5.71 ± 1.19	22.88 ± 5.81
Round 2				
All	606.11 ± 155.75	16.92 ± 5.05	6.81 ± 1.89	23.74 ± 5.81
15 cmH_2O	543.25 ± 170.26	16.80 ± 4.96	7.82 ± 2.04	24.62 ± 5.49
20 cmH_2O	656.40 ± 130.19	17.02 ± 5.38	6.01 ± 1.37	23.03 ± 6.25
Round 3				
All	595.00 ± 160.17	17.79 ± 5.86	6.88 ± 1.74	24.68 ± 6.39
15 cmH_2O	553.13 ± 162.81	16.08 ± 5.18	7.66 ± 1.85	23.75 ± 5.64
20 cmH_2O	628.50 ± 158.24	19.16 ± 6.27	6.26 ± 1.45	25.42 ± 7.14
Total				
All	606.76 ± 147.76	17.03 ± 4.63	6.70 ± 1.75	23.73 ± 5.27
15 cmH_2O	550.58 ± 163.48	16.35 ± 4.74	7.65 ± 1.93	24.01 ± 5.24
20 cmH_2O	651.70 ± 124.21	17.57 ± 4.72	5.95 ± 1.20	23.51 ± 5.57

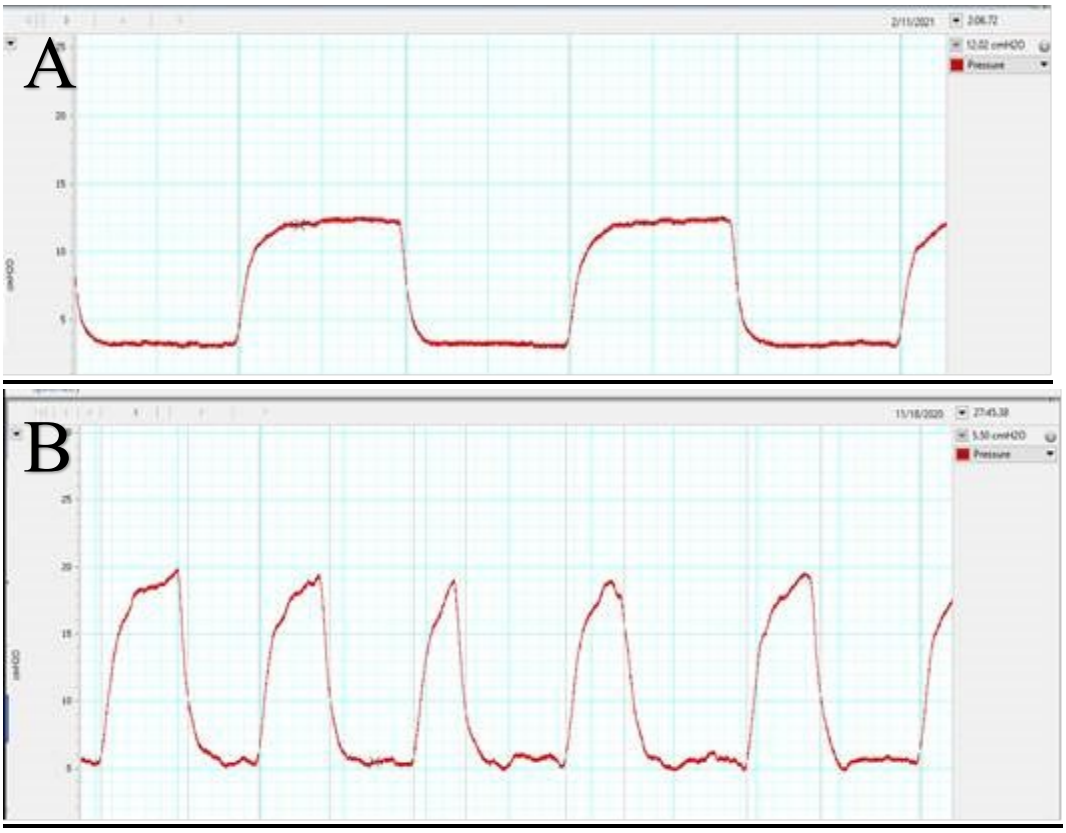


Figure 12. (A) Example of a subject that is breathing with the ventilator. Identified by a smooth pressure signal with a longer inspiratory duration, with a regular breath frequency. (B) Example of a subject that is fighting/resisting the ventilator. Identified by a choppier pressure signal, shorter inspiratory duration, likely not even a full breath with a higher breath frequency.

4.2.2 Inspiratory and Expiratory Muscle Fatigue

Over the course of the studies there were no evidence of inspiratory muscle fatigue observed within the subjects from baseline (107.96 ± 28.29 cmH_2O) to post 3 (107.27 ± 22.22 cmH_2O) measures (**Table 6**). There was also no evidence of expiratory muscle fatigue observed from baseline (132.65 ± 31.55 cmH_2O) to post 3 (126.87 ± 28.84 cmH_2O) measures.

Table 6. Muscle Fatigue. Mean ± SD

	Baseline	Post 3
MIPS (cmH_2O)		
All	107.96 ± 28.29	107.27 ± 22.22
15 cmH_2O	108.75 ± 31.00	109.25 ± 21.47
20 cmH_2O	107.09 ± 26.50	104.90 ± 24.04
MEPS (cmH_2O)		
All	132.65 ± 31.55	126.87 ± 28.84
15 cmH_2O	138.67 ± 32.58	129.67 ± 30.16
20 cmH_2O	126.09 ± 30.51	123.82 ± 28.46

4.2.3 Breathing patterns and gas exchange

For the respiratory gas exchange measures (**Table 7**), there was a significant decrease over time in VCO_2 ($p = 0.001$), Vt ($p=0.05$), RER ($p=0.001$), and $ETCO_2$ ($p=0.006$). There was a significant group x time interaction within SpO_2 ($p = 0.009$), however this does not appear to be clinically significant as both groups maintained > 98% average during all four measures (Baseline, Post 1, 2, 3). There was also an overall average decrease in HR ($p = 0.001$) over time, along with a significant increase in Fb ($p = 0.02$).

Table 7. Gas Exchange. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
VO_2 (mL/min)				
All	291.51 ± 60.85	284.55 ± 68.37	286.66 ± 75.28	288.05 ± 74.53
15 cmH_2O	289.70 ± 59.48	287.12 ± 76.67	298.03 ± 93.35	298.96 ± 92.38
20 cmH_2O	293.32 ± 64.79	281.98 ± 62.30	281.30 ± 54.60	277.14 ± 53.12
VCO_2 (mL/min)	***p=0.001 (time)**			
All	288.12 ± 62.51	241.61 ± 90.10	233.47 ± 78.44	228.25 ± 75.20
15 cmH_2O	289.43 ± 66.44	262.88 ± 100.04	253.97 ± 82.79	244.57 ± 75.96
20 cmH_2O	286.82 ± 61.25	220.35 ±77.32	212.98 ± 71.36	211.93 ± 73.99
Vt (mL)	***p=0.05 (time)**			
All	793.31 ± 294.13	722.60 ± 347.36	648.43 ± 261.60	688.15 ± 393.07
15 cmH_2O	842.90 ± 328.65	855.80 ± 300.50	728.06 ± 215.08	722.12 ± 225.02
20 cmH_2O	743.71 ± 259.82	589.41 ± 351.12	568.80 ± 288.09	654.18 ± 519.53
RER	***p=0.001 (time)**			
All	0.99 ± 0.11	0.84 ± 0.18	0.80 ± 0.16	0.79 ± 0.15
15 cmH_2O	1.00 ± 0.10	0.91 ± 0.19	0.86 ± 0.13	0.82 ± 0.12
20 cmH_2O	0.99 ± 0.12	0.77 ± 0.15	0.75 ± 0.17	0.75 ± 0.17
VE/VCO_2	***p=0.001 (time)**			
All	36.10 ± 2.94	42.10 ± 7.16	43.53 ± 7.27	43.93 ± 5.49
15 cmH_2O	36.48 ± 3.53	41.52 ± 5.88	41.44 ± 5.79	42.28 ± 5.61
20 cmH_2O	35.73 ± 2.31	42.68 ± 8.48	45.61 ± 8.22	45.59 ± 5.05
$ETCO_2$ (mmHg)	***p=0.006 (time)**			
All	34.41 ± 3.91	32.10 ± 4.65	32.03 ± 4.79	30.84 ± 4.87
15 cmH_2O	34.16 ± 3.62	30.84 ± 3.72	31.61 ± 4.42	30.24 ± 4.98
20 cmH_2O	34.67 ± 4.32	33.35 ± 5.29	32.45 ± 5.30	31.44 ± 4.90
HR (bpm)	***p=0.001 (time)**			
All	72.98 ± 8.97	65.18 ± 8.93	62.68 ± 10.04	62.99 ± 10.13
15 cmH_2O	75.45 ± 7.72	67.60 ± 8.58	66.29 ± 11.24	66.04 ± 11.18
20 cmH_2O	70.50 ± 9.75	62.76 ± 8.96	59.08 ± 7.50	59.93 ± 8.32
SpO_2	***p=0.009 (group)**			
All	99.27 ± 0.64	98.75 ± 1.51	99.13 ± 0.94	99.19 ± 0.94
15 cmH_2O	99.29 ± 0.54	99.17 ± 0.94	98.98 ± 0.94	98.79 ± 1.12
20 cmH_2O	99.25 ± 0.75	98.33 ± 1.88	99.27 ± 0.96	99.58 ± 0.52

4.2.4 Lung Mechanics: Reactance, Resistance, and Spirometry

In general resistance and reactance of peripheral and central airways measured with FOT at 5 Hz, 11 Hz and 19 Hz showed no significant difference over the course of the interventions (**Figure 13, 14; Table 8, 9, & 10**), however there was a significant increase over time for the reactance at 5 Hz Total ($p = 0.001$) suggesting potential some stiffening of the lower airways, along with a significant difference observed over time for R11 expiratory ($p = 0.001$) suggesting potentially some expiratory flow limitation, such as increases in airflow resistance of larger airways.

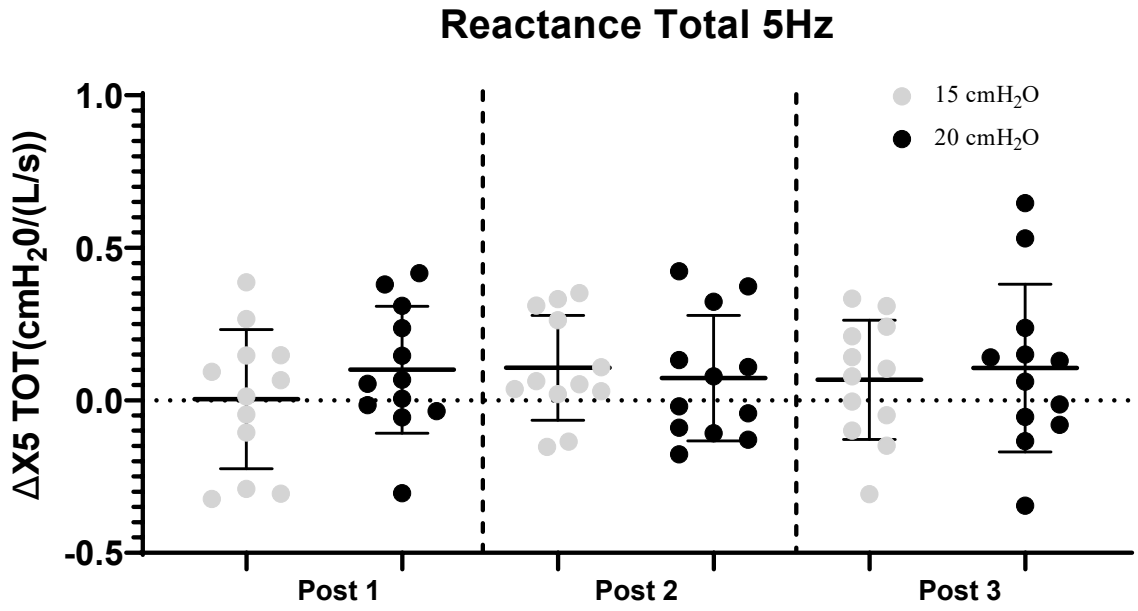


Figure 13. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for Reactance Total at 5Hz.

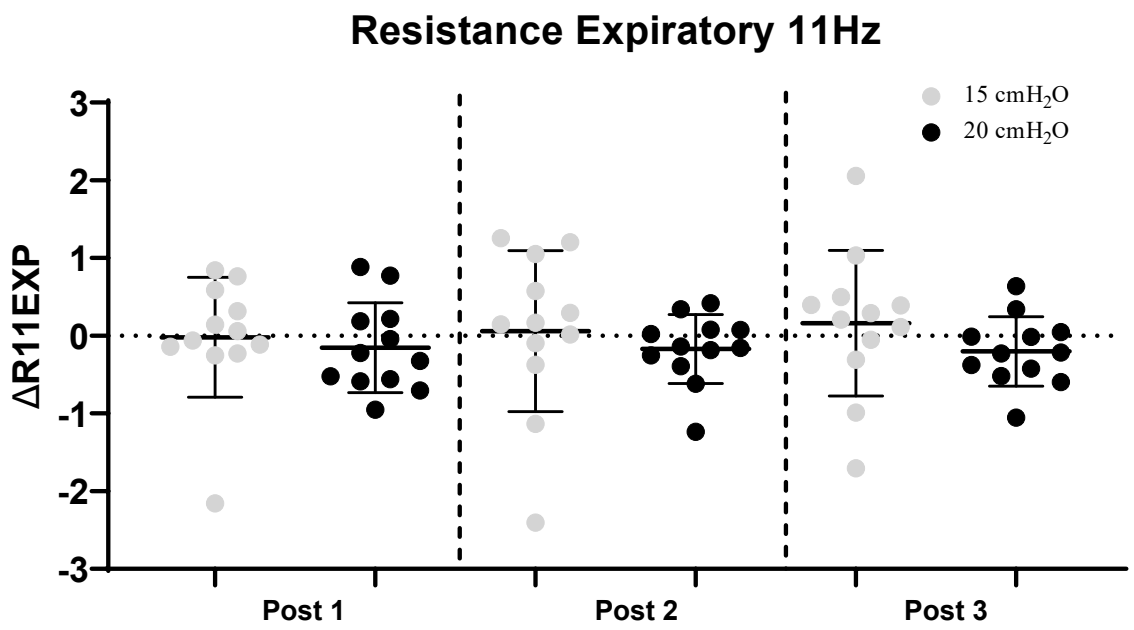


Figure 14. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for Resistance Expiratory at 11Hz.

Table 8. FOT 5Hz. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
R5 Inspiratory ($cmH_2O/(L/s)$)				
All	2.49 ± 0.70	2.46 ± 0.73	2.47 ± 0.75	2.47 ± 0.65
15 cmH_2O	2.43 ± 0.63	2.52 ± 0.69	2.56 ± 0.66	2.55 ± 0.64
20 cmH_2O	2.54 ± 0.79	2.39 ± 0.79	2.38 ± 0.84	2.40 ± 0.69
R5 Expiratory ($cmH_2O/(L/s)$)				
All	2.71 ± 0.97	2.59 ± 0.86	2.63 ± 0.90	2.68 ± 0.77
15 cmH_2O	2.81 ± 0.99	2.74 ± 0.79	2.81 ± 0.88	2.94 ± 0.78
20 cmH_2O	2.62 ± 0.98	2.44 ± 0.93	2.45 ± 0.92	2.42 ± 0.69
R5 Total ($cmH_2O/(L/s)$)				
All	2.63 ± 0.84	2.53 ± 0.79	2.56 ± 0.81	2.59 ± 0.66
15 cmH_2O	2.66 ± 0.84	2.66 ± 0.75	2.70 ± 0.75	2.77 ± 0.64
20 cmH_2O	2.59 ± 0.88	2.40 ± 0.85	2.41 ± 0.87	2.41 ± 0.66
X5 Inspiratory ($cmH_2O/(L/s)$)				
All	-0.82 ± 0.32	-0.76 ± 0.28	-0.74 ± 0.36	-0.74 ± 0.38
15 cmH_2O	-0.79 ± 0.25	-0.77 ± 0.26	-0.71 ± 0.37	-0.73 ± 0.37
20 cmH_2O	-0.85 ± 0.38	-0.75 ± 0.31	-0.76 ± 0.36	-0.75 ± 0.40
X5 Expiratory ($cmH_2O/(L/s)$)				
All	-0.73 ± 0.25	-0.66 ± 0.27	-0.65 ± 0.29	-0.66 ± 0.23
15 cmH_2O	-0.72 ± 0.21	-0.67 ± 0.25	-0.61 ± 0.23	-0.69 ± 0.20
20 cmH_2O	-0.74 ± 0.30	-0.64 ± 0.29	-0.69 ± 0.34	-0.64 ± 0.27
X5 Total ($cmH_2O/(L/s)$)	***p=0.001 (time)**			
All	-0.65 ± 0.62	-0.60 ± 0.55	-0.56 ± 0.63	-0.57 ± 0.62
15 cmH_2O	-0.52 ± 0.81	-0.51 ± 0.72	-0.41 ± 0.82	-0.45 ± 0.83
20 cmH_2O	-0.79 ± 0.32	-0.69 ± 0.29	-0.72 ± 0.32	-0.69 ± 0.31

Table 9. FOT 11Hz. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
R11 Inspiratory ($cmH_2O/(L/s)$)				
All	2.36 ± 0.63	2.36 ± 0.62	2.38 ± 0.66	2.39 ± 0.58
15 cmH_2O	2.34 ± 0.59	2.44 ± 0.59	2.53 ± 0.62	2.52 ± 0.58
20 cmH_2O	2.37 ± 0.70	2.29 ± 0.65	2.23 ± 0.68	2.27 ± 0.56
R11 Expiratory ($cmH_2O/(L/s)$)	***p=0.001 (time)**			
All	2.68 ± 0.90	2.59 ± 0.81	2.62 ± 0.84	2.66 ± 0.76
15 cmH_2O	2.79 ± 0.94	2.77 ± 0.78	2.85 ± 0.87	2.95 ± 0.75
20 cmH_2O	2.57 ± 0.88	2.41 ± 0.83	2.40 ± 0.77	2.37 ± 0.66
R11 Total ($cmH_2O/(L/s)$)				
All	2.47 ± 0.91	2.42 ± 0.86	2.42 ± 0.89	2.44 ± 0.82
15 cmH_2O	2.46 ± 1.06	2.49 ± 1.00	2.52 ± 1.06	2.55 ± 1.01
20 cmH_2O	2.49 ± 0.77	2.35 ± 0.73	2.32 ± 0.72	2.32 ± 0.59
X11 Inspiratory ($cmH_2O/(L/s)$)				
All	0.14 ± 0.30	0.18 ± 0.27	0.16 ± 0.26	0.17 ± 0.24
15 cmH_2O	0.20 ± 0.15	0.18 ± 0.21	0.19 ± 0.19	0.20 ± 0.20
20 cmH_2O	0.07 ± 0.40	0.18 ± 0.33	0.13 ± 0.32	0.14 ± 0.28
X11 Expiratory ($cmH_2O/(L/s)$)				
All	0.04 ± 0.33	0.09 ± 0.34	0.11 ± 0.29	0.07 ± 0.31
15 cmH_2O	0.01 ± 0.34	0.05 ± 0.29	0.11 ± 0.25	0.04 ± 0.25
20 cmH_2O	0.07 ± 0.33	0.13 ± 0.39	0.11 ± 0.34	0.10 ± 0.36
X11 Total ($cmH_2O/(L/s)$)				
All	0.08 ± 0.29	0.13 ± 0.29	0.13 ± 0.26	0.11 ± 0.25
15 cmH_2O	0.08 ± 0.25	0.10 ± 0.24	0.14 ± 0.20	0.11 ± 0.19
20 cmH_2O	0.08 ± 0.34	0.16 ± 0.34	0.12 ± 0.32	0.12 ± 0.30

Table 10. FOT 19Hz. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
R19 Inspiratory ($cmH_2O/(L/s)$)				
All	2.40 ± 0.73	2.47 ± 0.70	2.44 ± 0.82	2.47 ± 0.78
15 cmH_2O	2.36 ± 0.83	2.53 ± 0.79	2.52 ± 0.97	2.53 ± 0.95
20 cmH_2O	2.43 ± 0.64	2.42 ± 0.63	2.36 ± 0.67	2.40 ± 0.60
R19 Expiratory ($cmH_2O/(L/s)$)				
All	2.64 ± 0.93	2.63 ± 0.80	2.65 ± 0.92	2.67 ± 0.85
15 cmH_2O	2.66 ± 1.03	2.74 ± 0.86	2.80 ± 1.08	2.87 ± 1.00
20 cmH_2O	2.61 ± 0.86	2.52 ± 0.76	2.50 ± 0.75	2.48 ± 0.66
R19 Total ($cmH_2O/(L/s)$)				
All	2.55 ± 0.83	2.56 ± 0.75	2.56 ± 0.87	2.59 ± 0.79
15 cmH_2O	2.55 ± 0.94	2.66 ± 0.83	2.69 ± 1.02	2.73 ± 0.94
20 cmH_2O	2.54 ± 0.74	2.47 ± 0.70	2.44 ± 0.71	2.44 ± 0.62
X19 Inspiratory ($cmH_2O/(L/s)$)				
All	1.03 ± 0.46	1.05 ± 0.45	1.03 ± 0.41	1.02 ± 0.36
15 cmH_2O	1.04 ± 0.37	1.01 ± 0.41	1.01 ± 0.38	1.00 ± 0.34
20 cmH_2O	1.02 ± 0.56	1.10 ± 0.51	1.05 ± 0.45	1.03 ± 0.40
X19 Expiratory ($cmH_2O/(L/s)$)				
All	0.72 ± 0.47	0.78 ± 0.53	0.80 ± 0.44	0.76 ± 0.45
15 cmH_2O	0.65 ± 0.45	0.66 ± 0.44	0.75 ± 0.36	0.66 ± 0.37
20 cmH_2O	0.80 ± 0.50	0.89 ± 0.61	0.85 ± 0.52	0.85 ± 0.52
X19 Total ($cmH_2O/(L/s)$)				
All	0.85 ± 0.45	0.90 ± 0.47	0.90 ± 0.41	0.87 ± 0.38
15 cmH_2O	0.81 ± 0.38	0.80 ± 0.40	0.86 ± 0.34	0.80 ± 0.30
20 cmH_2O	0.89 ± 0.52	1.00 ± 0.53	0.94 ± 0.48	0.93 ± 0.45

The spirometry measures (**Table 11**) showed a significant group × time interaction for FEF50/FIF50 ($p = 0.013$). With the 20 cmH_2O group having a larger average decrease of 20.83% from baseline to post 3 compared to the 15.2% observed within the 15 cmH_2O group. There was also a significant group × time interaction for FIF Max ($p = 0.004$), were as the 20 cmH_2O group had a greater change (1.13L/sec) from baseline (4.04 ± 1.21) to post 3 (5.17 ± 2.04), compared to the change (-0.07L/sec) seen within the 15 cmH_2O group from baseline (4.70 ± 2.08) to post 3 (4.63 ± 2.28). The spirometry measures also showed a significant average decrease over time for SVC ($p = 0.016$), along with a significant change over time for MVV ($p = 0.012$ and MVV Vt ($p = 0.05$). However, there was no significant difference observed with in the IC, FVC (**Figure 15**), FEV1, FEF25-75 (**Figure 16)**, FEF Max, FIVC or the MVV RR measures.

Table 11. Spirometry. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
SVC (L)	***p=0.016 (time)**			
All	4.73 ± 1.07	4.59 ± 1.00	4.60 ± 0.96	4.63 ± 1.03
15 cmH₂O	4.69 ± 1.31	4.51 ± 1.26	4.53 ± 1.20	4.49 ± 1.20
20 cmH₂O	4.76 ± 0.83	4.67 ± 0.69	4.66 ± 0.70	4.77 ± 0.86
IC (L)				
All	3.29 ± 0.81	3.12 ± 0.72	3.16 ± 0.76	3.24 ± 0.79
15 cmH₂O	3.34 ± 1.01	3.10 ± 0.86	3.17 ± 0.91	3.21 ± 0.85
20 cmH₂O	3.24 ± 0.58	3.14 ± 0.58	3.14 ± 0.60	3.27 ± 0.77
FVC (L)				
All	4.80 ± 1.05	4.72 ± 0.96	4.76 ± 0.98	4.80 ± 1.05
15 cmH₂O	4.76 ± 1.28	4.67 ± 1.22	4.70 ± 1.19	4.69 ± 1.25
20 cmH₂O	4.84 ± 0.81	4.77 ± 0.66	4.82 ± 0.76	4.91 ± 0.85
FEV1 (L)				
All	3.89 ± 0.89	3.81 ± 0.87	3.85 ± 0.90	3.92 ± 0.97
15 cmH₂O	3.83 ± 1.07	3.74 ± 1.09	3.76 ± 1.07	3.81 ± 1.14
20 cmH₂O	3.96 ± 0.70	3.88 ± 0.61	3.95 ± 0.73	4.04 ± 0.81
FEF 25-75 (L/sec)				
All	3.84 ± 1.26	3.72 ± 1.31	3.79 ± 1.48	3.96 ± 1.57
15 cmH₂O	3.67 ± 1.15	3.59 ± 1.37	3.56 ± 1.38	3.71 ± 1.43
20 cmH₂O	4.02 ± 1.38	3.86 ± 1.30	4.02 ± 1.59	4.21 ± 1.72
FEF Max (L/sec)				
All	8.61 ± 2.21	8.49 ± 2.15	8.50 ± 2.07	8.94 ± 2.43
15 cmH₂O	8.90 ± 2.30	8.68 ± 2.42	8.68 ± 2.32	8.74 ± 2.49
20 cmH₂O	8.33 ± 2.18	8.30 ± 1.95	8.31 ± 1.88	9.14 ± 2.46
FIVC (L)				
All	3.63 ± 0.89	3.49 ± 1.16	3.64 ± 0.86	3.72 ± 0.96
15 cmH₂O	3.61 ± 1.04	3.69 ± 1.12	3.61 ± 0.90	3.81 ± 1.01
20 cmH₂O	3.66 ± 0.75	3.39 ± 1.21	3.67 ± 0.86	3.64 ± 0.94
FEF50/FIF50 (%)	***p=0.013 (group)**			
All	128.58 ± 59.60	114.33 ± 50.77	127.50 ± 59.13	113.38 ± 47.70
15 cmH₂O	122.83 ± 64.57	116.75 ± 57.80	147.17 ± 58.65	113.25 ± 37.70
20 cmH₂O	134.33 ± 56.45	110.09 ± 46.86	107.83 ± 55.01	113.50 ± 57.75
FIF Max (L/sec)	***p=0.004 (group)**			
All	4.37 ± 1.70	4.61 ± 1.83	4.42 ± 1.94	4.90 ± 2.14
15 cmH₂O	4.70 ± 2.08	4.43 ± 1.83	3.70 ± 1.74	4.63 ± 2.28
20 cmH₂O	4.04 ± 1.21	4.91 ± 1.93	5.15 ± 1.92	5.17 ± 2.04
MVV (L)	***p=0.012 (time)**			
All	146.00 ± 42.23	141.96 ± 40.21	144.46 ± 35.90	155.17 ± 37.73
15 cmH₂O	144.50 ± 40.07	145.25 ± 40.52	143.92 ± 35.75	154.75 ± 36.95
20 cmH₂O	147.50 ± 46.03	142.67 ± 40.79	145.00 ± 37.62	155.58 ± 40.13
MVV Vt (L)	***p=0.05 (time)**			
All	2.05 ± 0.84	1.94 ± 0.79	1.94 ± 0.74	2.16 ± 0.90
15 cmH₂O	2.11 ± 0.74	2.00 ± 0.64	2.03 ± 0.65	2.15 ± 0.58
20 cmH₂O	2.00 ± 0.97	1.92 ± 0.97	1.86 ± 0.84	2.17 ± 1.17
MVV RR (bpm)				
All	76.38 ± 18.82	79.35 ± 21.94	80.85 ± 22.09	79.39 ± 20.41
15 cmH₂O	72.39 ± 19.02	75.42 ± 16.00	74.93 ± 18.71	77.24 ± 15.65
20 cmH₂O	80.37 ± 18.55	84.60 ± 26.92	86.76 ± 24.37	81.54 ± 24.83

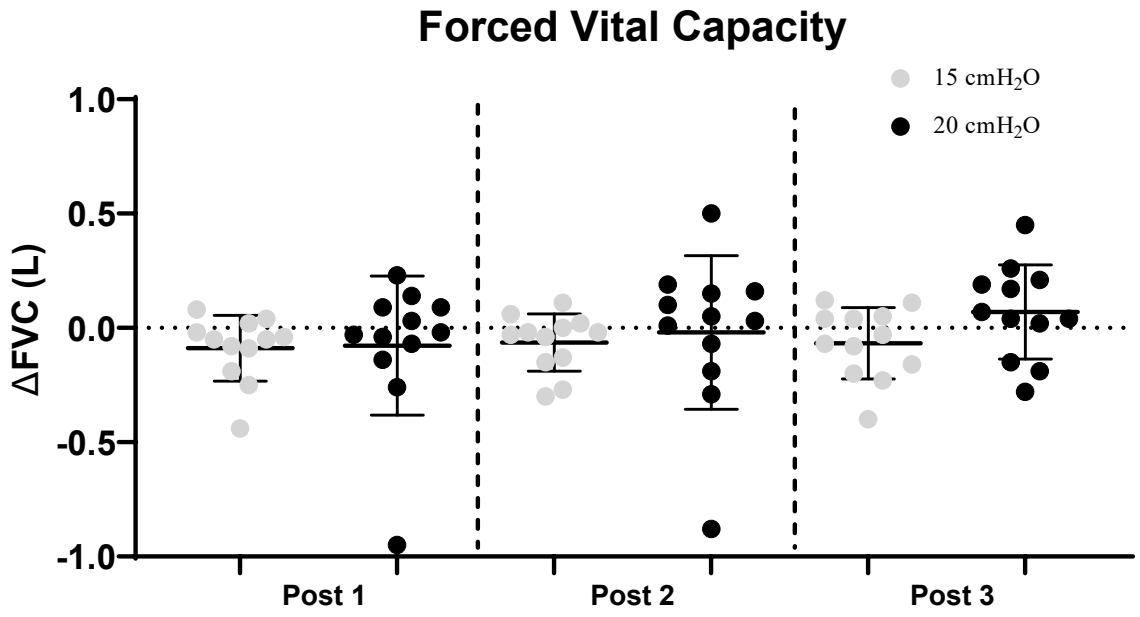


Figure 15. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for Forced Vital Capacity.

*Outlier at Post 1 and 2 had baseline measure of 6.83 L and was higher at post 3 at 6.68L, suggesting potentially poor effort rather than transient decline in vital capacity.

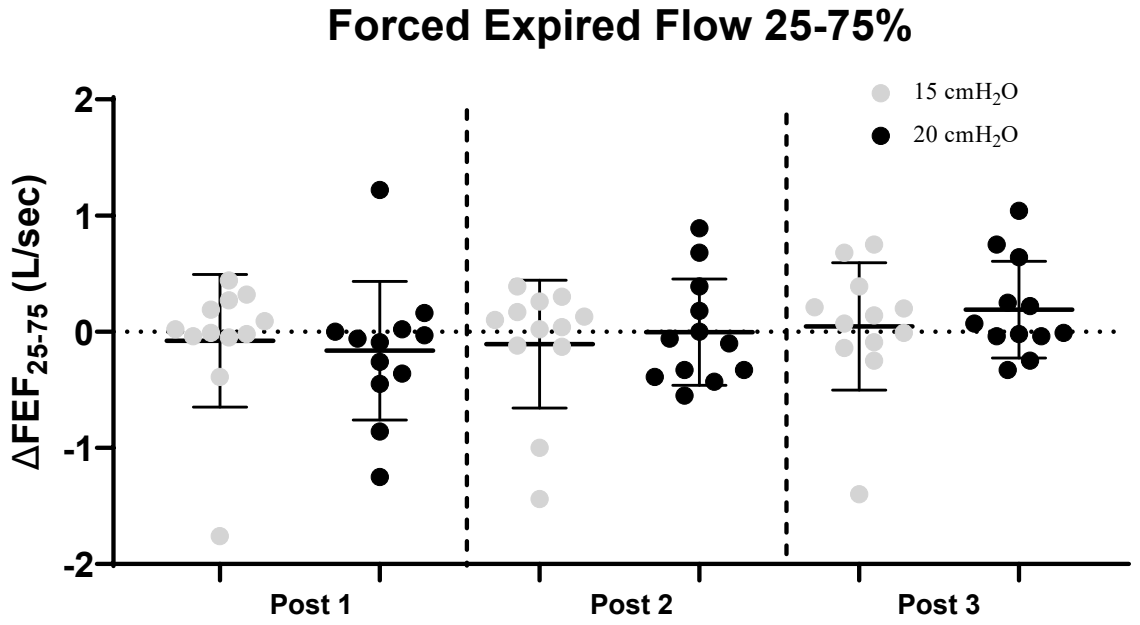


Figure 16. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for FEF25-75.

There was no significant change in ExNO with positive pressure breathing in the presence of an elastic load with repeat loading or between groups (**Figure 17; Table 12**),

Table 12. Exhaled Nitric Oxide. Mean ± SD

	Baseline	Post 1	Post 2	Post 3
ExNO				
All	13.50 ± 8.16	13.63 ± 6.95	12.96 ± 6.58	12.75 ± 6.41
15 cmH_2O	14.25 ± 9.55	15.00 ± 7.17	14.00 ± 7.05	12.83 ± 6.97
20 cmH_2O	12.75 ± 6.84	12.25 ± 6.74	11.92 ± 6.22	12.67 ± 6.11

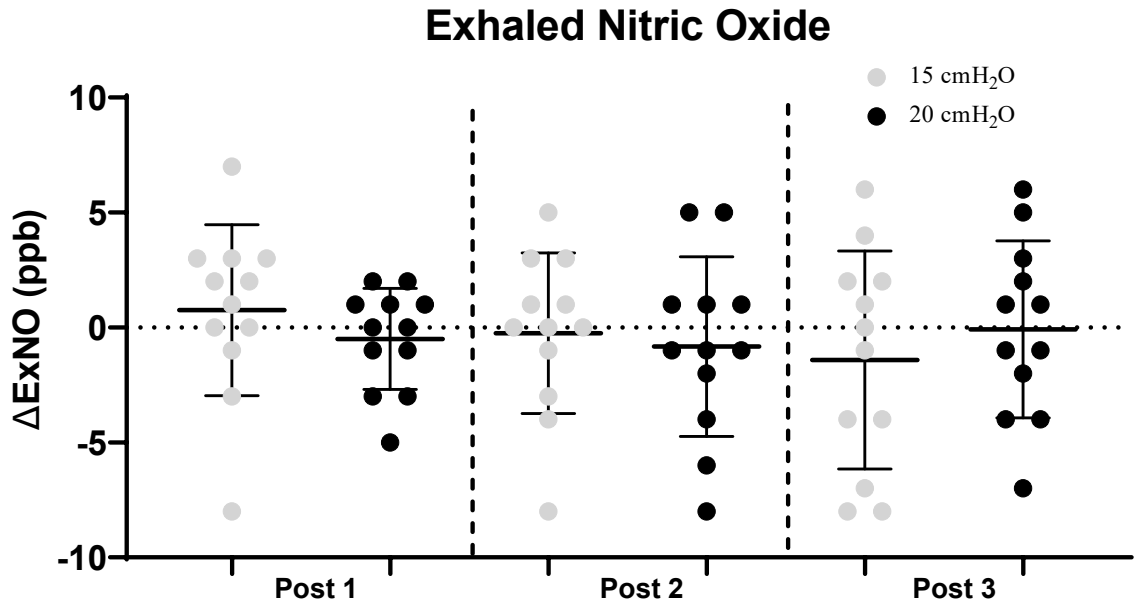


Figure 17. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for exhaled nitric oxide.

4.2.5 Hemodynamics and Pulmonary Vasculature

There was a significant change over time for cardiac output (Q, p = 0.018, **Figure 18, Table 13**) and alveolar capillary recruitment (DLCO/Q, p = 0.007, **Figure 19**), but no difference between groups. There were no significant changes in the lung gas transfer across the alveolar

capillary membrane (DLCO) or the surface area for gas exchange in the lungs (DM), or functional unit of membrane diffusion relative to capillary blood volume (DM/Vc) with repeated positive pressure breathing under elastic load and between groups ($p>0.05$, (**Figure 20-22**) measures. The comet tail measures (**Table 14**) made during post 3 showed no significant difference from the baseline measures suggesting minimal change in lung fluid balance from intra to extravascular.

Table 13. Lung Diffusion and Cardiac Output. Mean ± SD

	Baseline	**Post 1**	**Post 2**	**Post 3**
DLCO (mL/min/mmHg)				
All	24.60 ± 7.10	24.94 ± 7.09	23.56 ± 6.76	24.59 ± 6.99
15 cmH_2O	24.83 ± 8.40	24.49 ± 8.44	22.77 ± 7.69	23.76 ± 7.95
20 cmH_2O	24.39 ± 6.05	25.40 ± 5.81	24.30 ± 6.03	25.36 ± 6.23
DM (mL/min/mmHg)				
All	34.38 ± 12.24	32.74 ± 9.28	30.54 ± 9.12	32.84 ± 10.58
15 cmH_2O	37.93 ± 15.24	33.03 ± 11.39	31.37 ± 12.13	31.79 ± 11.54
20 cmH_2O	31.12 ± 8.03	32.46 ± 7.14	29.86 ± 6.25	33.81 ± 10.03
Q (L/min)	***p=0.018 (time)**			
All	5.95 ± 1.42	5.19 ± 1.43	5.04 ± 1.10	5.58 ± 2.12
15 cmH_2O	5.70 ± 1.61	5.10 ± 1.19	4.77 ± 1.09	5.35 ± 2.80
20 cmH_2O	6.18 ± 1.25	5.27 ± 1.67	5.28 ± 1.09	5.80 ± 1.35
Vc (mL)				
All	217.88 ± 117.64	209.12 ± 82.69	209.76 ± 89.62	242.58 ± 160.37
15 cmH_2O	164.31 ± 34.26	196.38 ± 59.42	176.35 ± 56.58	214.08 ± 102.16
20 cmH_2O	261.70 ± 143.74	220.58 ± 101.14	239.83 ± 105.35	265.90 ± 197.96
DLCO/Q (L/min/mmHg)	***p=0.007 (time)**			
All	4.24 ± 1.03	4.74 ± 1.02	4.75 ± 1.02	4.62 ± 1.03
15 cmH_2O	4.36 ± 0.75	4.74 ± 0.85	4.74 ± 0.97	4.79 ± 1.19
20 cmH_2O	4.14 ± 1.25	4.73 ± 1.22	4.76 ± 1.12	4.47 ± 0.88
DLCO/VA (L/min/mmHg)				
All	5.95 ± 1.23	5.79 ± 1.41	5.45 ± 1.26	5.89 ± 1.35
15 cmH_2O	6.08 ± 1.19	5.76 ± 1.50	5.44 ± 1.27	5.74 ± 1.40
20 cmH_2O	5.84 ± 1.31	5.81 ± 1.40	5.47 ± 1.31	6.02 ± 1.34
DM/Vc				
All	0.18 ± 0.08	0.17 ± 0.08	0.20 ± 0.12	0.17 ± 0.09
15 cmH_2O	0.22 ± 0.07	0.18 ± 0.09	0.23 ± 0.13	0.17 ± 0.09
20 cmH_2O	0.15 ± 0.08	0.16 ± 0.07	0.18 ± 0.12	0.18 ± 0.11

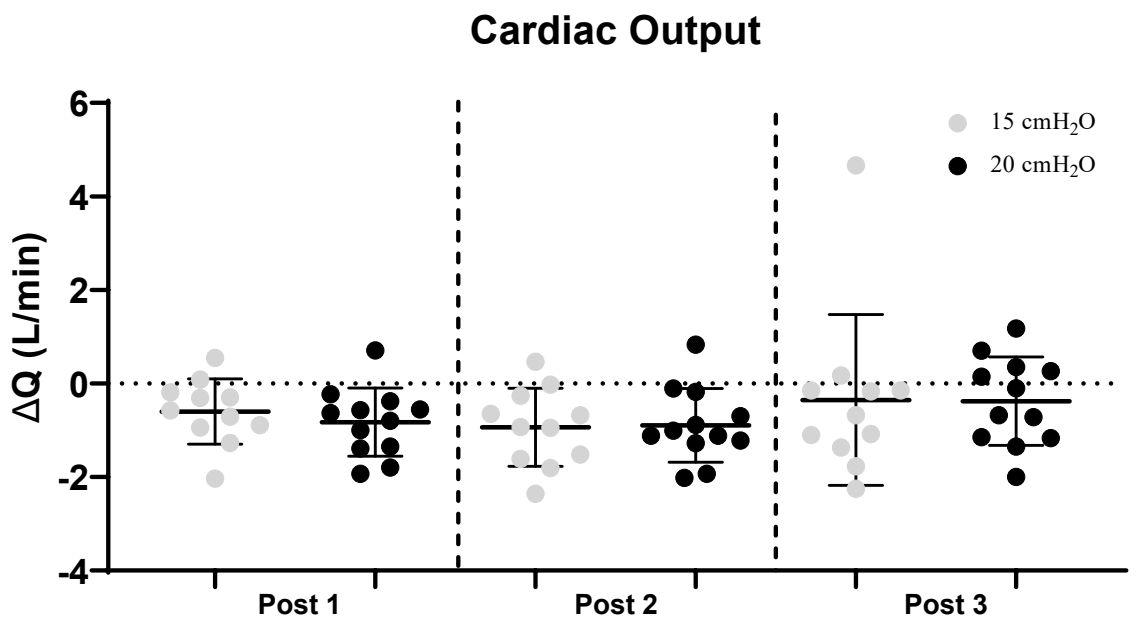


Figure 18. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for cardiac output.

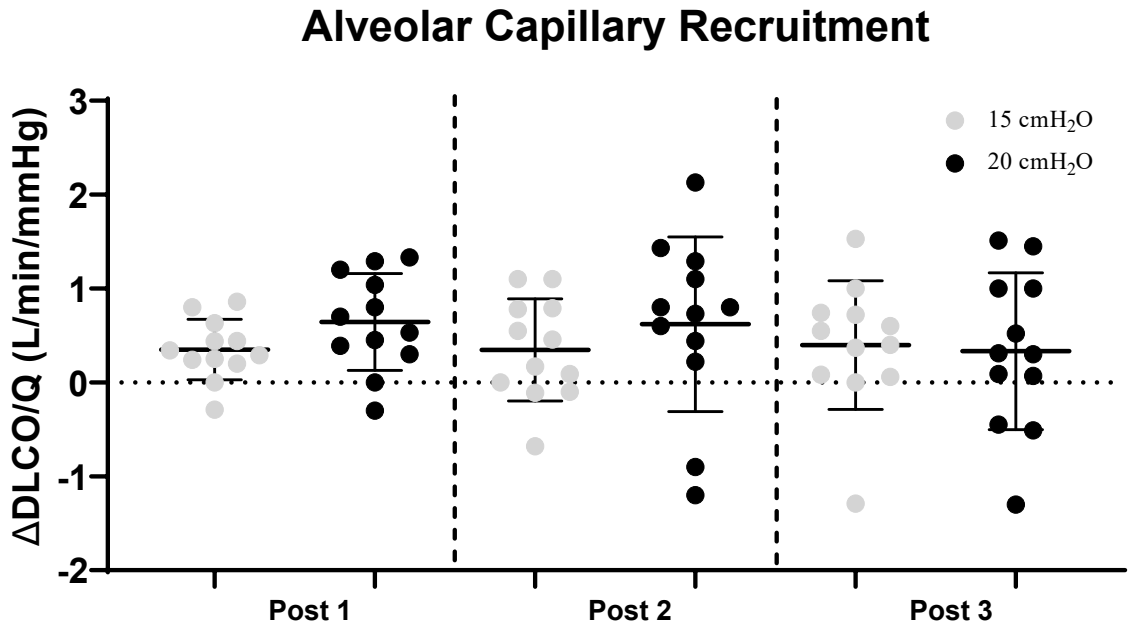


Figure 19. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for alveolar capillary recruitment.

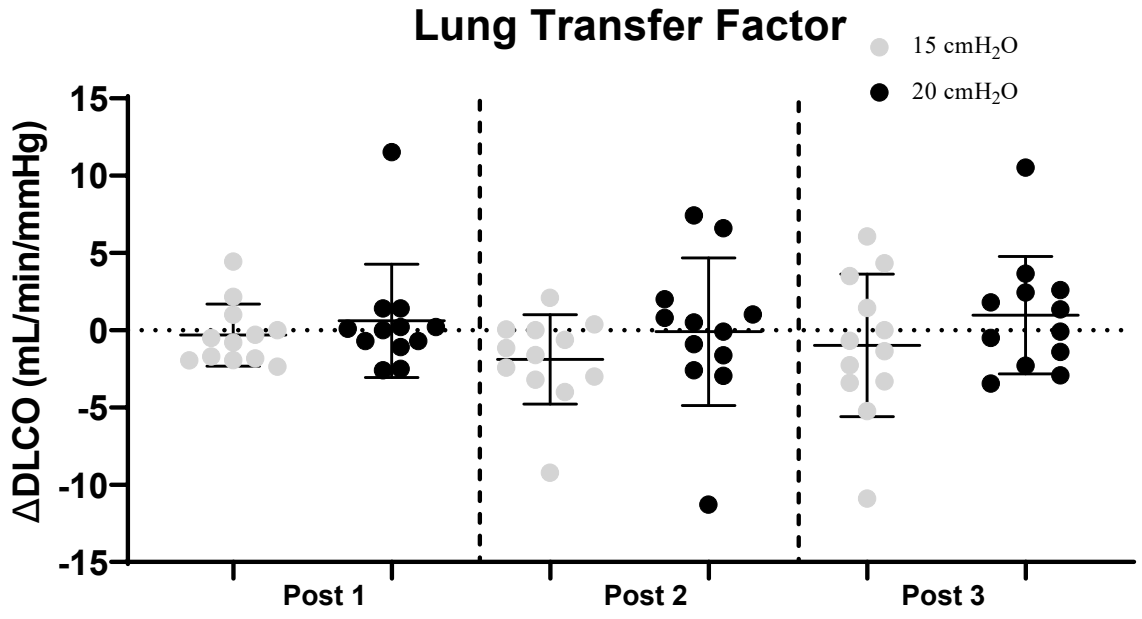


Figure 20. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for lung transfer factor.

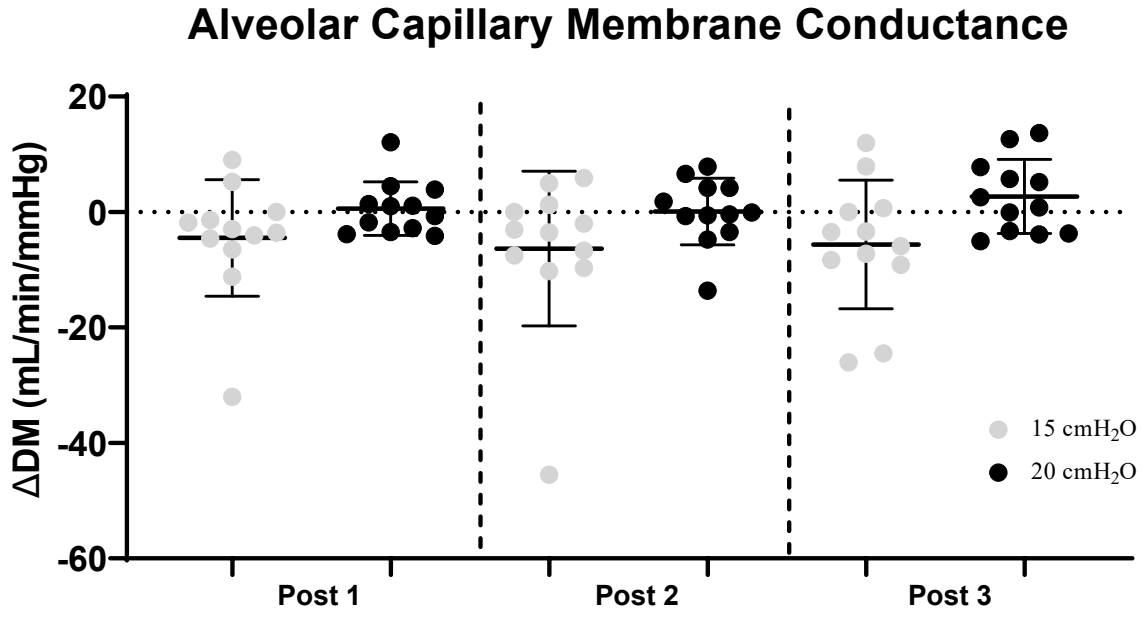


Figure 21. Change from baseline measures across the three, post positive pressure breathing with elastic loading ventilator testing sessions for alveolar capillary membrane conductance.

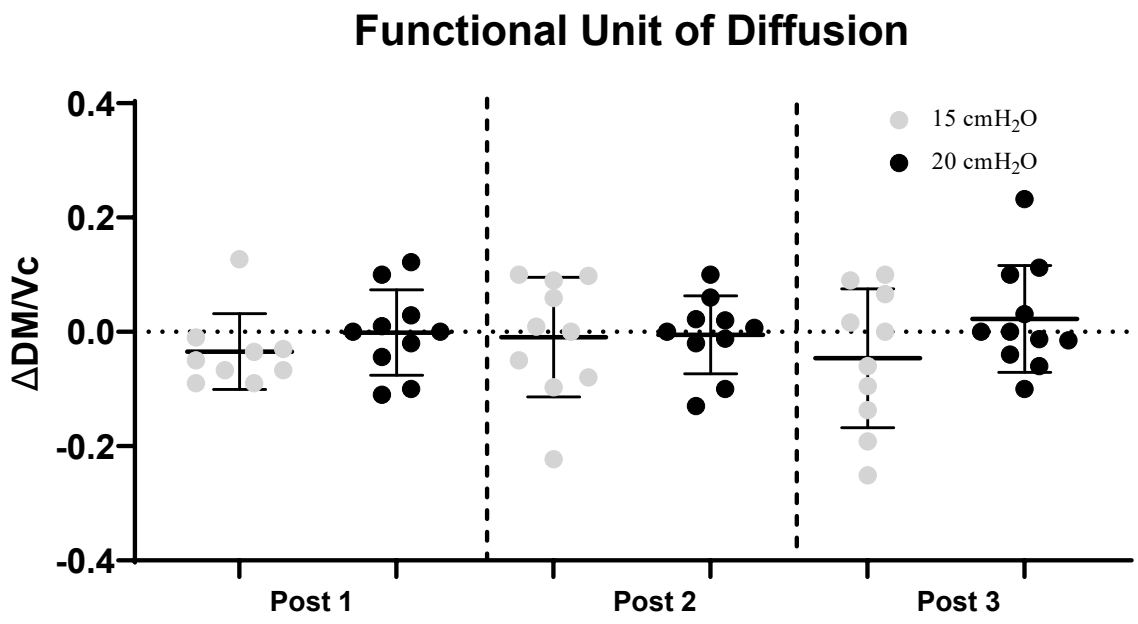


Figure 22. Change from baseline measures across the three, post positive pressure breathing with elastic loading sessions for functional unit of diffusion.

Table 14. Comet Tails. Mean ± SD

	Baseline	Post 3
All	2.75 ± 3.47	3.08 ± 3.28
15 cmH_2O	2.50 ± 3.40	3.25 ± 4.18
20 cmH_2O	3.00 ± 3.67	2.92 ± 2.23

4.3 Study Aim 1 Discussion

On average, performing three sequential 30 min bouts of inspiratory threshold loading at 40 and 60% of MIPS breathing at rest at 15 breaths per minute and 60% duty cycle resulted in minimal changes in gas exchange, respiratory mechanics, lung physiology, and measures of lung injury in relatively young healthy adults. When assessing resting non-loaded gas exchange measures after each bout, there was an increase in RR and VE/VCO_2 with a decreases in VT, VCO_2, VE and $ETCO_2$ indicating that subjects are experiencing some alterations in metabolic

demand, ventilatory control and gas exchange following repeated inspiratory loading. It is unclear what exactly lead to the increases in RR and VE/VCO_2 with decreases in VT, VCO_2, VE, and $ETCO_2$. However, we can speculate that the decrease in VT may suggest that subjects were becoming fatigued from the repeated inspiratory loading and taking smaller breaths to reduce the respiratory work, and despite increasing RR still resulted in reductions in VE. The increase in VE/VCO_2 for both groups with repeated inspiratory loading may have been caused by over breathing or ventilating at a higher rate than required for CO_2 production. This was also accompanied with the decreases in VCO_2 and $ETCO_2$ supporting the notion that subjects are hyperventilating and blowing off CO_2.

The nonsignificant changes in resistance and reactance indicates that repeated inspiratory loading is not impacting the lungs ability to distend and recoil either centrally or peripherally. Additionally, the nonsignificant changes in reactance would indicate that the distant airways are being ventilated appropriately. Although, there were declines in SVC, FVC, and FEV_1 with repeated inspiratory loading there were no changes in resistance and reactance measured with FOT. Thus, the decline in SVC, FVC, FEV_1 may be more a result of respiratory muscle fatigue than indicative of pathophysiological alterations in the lungs.

Subjects in the 60% MIPS group performed a higher total respiratory muscle work based on total PTP compared to subjects in the 40% MIPS group, however there were no differences between the groups for any measure of lung physiology, lung function, or lung injury. The total PTP was significantly and negatively correlated with the DLCO/Q, indicating that higher levels of respiratory muscles effort or work during inspiration can potentially start to affect alveolar-capillary recruitment.

The findings from this study found trends that indicate inspiratory loaded breathing can lead to respiratory muscle fatigue, but minimal evidence of significant decrements in lung physiology that would negatively impact gas exchange. Subjects were exposed to a very controlled environment and only had to focus on breathing against an inspiratory load which they tolerated on average for 19-22 minutes per round. Pilots, however, will not be exposed to a highly controlled environment with only one task to focus on. The amount of respiratory loading, the time under tension, and the conditions in which the subject experiences the load either stressed or resting will impact how respiratory loading affects lung function and physiology.[21] Under extremely stressful conditions (high inspiratory loads or overt occlusion along with hypoxia – anxiety) it is possible for neurogenic forms of pulmonary edema to develop. The methodology and findings of this study are relevant to military pilots because they are subjected to dramatic changes in cabin pressure with fluctuations in altitude, which require them to breath with an OBOGS. The OBOGS may increase the work and cost of breathing due to an increased inspiratory load. The degree to which pilot's lung physiology and function will be impacted will depend on the amount of time breathing with the OBOGS, the amount of resistance generated by the OBOGS, and the additional environmental stressors the pilot is experiencing. Based on the findings of this study, repeated bouts of inspiratory loading under normal resting conditions may cause respiratory muscle fatigue and small declines in lung volumes (i.e., SVC, FVC and FEV_1).

Based on the data collected in the current study the resting challenge of loaded inspiratory breathing was not enough to cause changes in lung physiology that would functionally and negatively alter respiratory gas exchange in healthy young adults. There may be mild and transient changes in breathing pattern and mild hyperventilation associated with the loads that could impact cerebral blood flow, although these changes were also mild. Additionally, in this study there was

no evidence of developing lung or airway inflammation or alterations in lung surface area for gas exchange or alterations in lung fluid balance or pulmonary vascular permeability. Future studies could consider more variable loads, expiratory loads or combining multiple stressors such as the combination of inspiratory loaded breathing with an increase in metabolic demand as well as exposure to high oxygen levels and dry gases. Military pilots breathe against an inspiratory load as well as receive high concentrations of oxygen and as such adding stressors sequentially may help determine the combination of factors that negatively impact lung physiology and function. Furthermore, increasing metabolic demand by having subjects complete a submaximal activity while breathing with an inspiratory load will further reduce the ability of subjects to attempt to "rest" or minimize work between breaths, enhance the sympathetic nervous system response to the load and increase the competition of blood flow and metaboreflex associated with respiratory muscle fatigue [22]. As such, it is necessary to explore how increasing the work between breaths as well as adding stressors sequentially will impact lung function and lung physiology. By creating an environment that more closely resembles what pilots are exposed to, we will be able to gain a greater understanding of how the respiratory system will be impacted acutely.

4.4 Study Aim 2 Discussion

The primary purpose of this study was to examine the influence of positive pressure breathing while exposed to an elastic load on measures of lung physiology in healthy individuals. The current findings indicated the elastic wrapping caused a significant FVC reduction (22.41%), however, in combination with the positive pressure ventilator sessions a significant average increase in Vt (52%) and Fb (36%) was observed compared to the average unwrapped testing sessions. However, with these simultaneous interventions there were no significant change in lung inflammation as measured by ExNO. There were, however, some significant decreases observed

over time within some of the gas exchange measures (measured post each exposure, VCO_2, Vt, RER, and $ETCO_2$) due to the elastic loading and positive pressure ventilator sessions, but they do not appear to provide any change in lung physiology that might compromise normal function.

Interestingly, a previous research study showed Vt to be unaltered during thoracic restriction [12], but this could be due to the less restriction being applied within the study (5%), in comparison to the 22.41% within the current study. But the researchers did suggest that by applying an elastic load caused the respiratory muscles to compensate by increasing neural excitation to overcome the load in order to meet the respiratory drive - demand [12].

Both chest wall elastic loading and positive pressure ventilation by themselves have shown to cause lung decrements and reduced function, but utilized together, such as the systems in place within high performance aircraft and it's pilot, it appears a synergistic relationship is made that likely reduces the inflation influence of the OBOGS but generally allows efficient gas exchange within these high performance pilots, during strenuous, G-force creating maneuvers. Thus the elastic loading maybe protecting the lung from over stretching and other trauma that could be caused by (in our case) the ventilator, by forcing individuals to breath a lower lung volumes and resisting the positive pressure and given the lack of G – forces and high O_2, the risk of atelectasis and the need to be able to reverse the atelectasis with larger breaths which would have been more difficult with an elastic load.

5.0 SUMMARY and CONCLUSIONS

5.1 Study Aim 1

Pilots are likely exposed to various levels of inspiratory muscle loading during flight and we estimated that loads of 40 – 60% or greater of individual maximal inspiratory pressure generation may be common. However, previous studies have suggested that heavy, fatiguing

inspiratory loads may alter transmural pressures the lungs are exposed to and may ultimately alter airway tissue and lung parenchyma, causing inflammation and altered permeability of the pulmonary vasculature making it susceptible to altered lung water in the extravascular space – which could ultimately influence gas transfer, lung compliance and lung volumes and flows.

In this study we exposed healthy individuals to inspiratory loads of approximately 40 and 60% of their individual *Maximal Inspiratory Pressure* generating capacity during resting breathing. Subjects were given visual feedback with the goal of maintaining a pressure target over the entire inspiratory cycle phase to either exhaustion (inability to reach target) or over 3 x 30 min exposures with measures of lung physiology before and after each exposure. On average, study subjects demonstrated evidence of inspiratory muscle fatigue of similar magnitude from both loads, although for the 60% loaded breathing, subjects could not maintain the load as long. Additionally, we observed small changes in lung mechanics and gas exchange as well as ventilatory control post exposures. The transient changes in breathing pattern and gas exchange between or after 3 bouts, are likely due to the inspiratory muscle fatigue as well as mild hyperventilation, rather than negative consequences related to altered lung mechanics or gas exchange. There were some small trends in worsening measures of gas transfer relative to the cumulative inspiratory load exposure, but not to the extent that would be clinically significant (DLCO/Q vs total respiratory load exposure). In the 24 study subjects, one subject demonstrated more significant decreases in gas transfer (˜4% of subjects), implying there may be some degree of susceptibility in some subjects.

5.2 Study Aim 2

Within this study, the applied elastic load caused a significant FVC reduction of 22.41%, and in combination with positive pressure ventilation caused a significant average increase in Vt (52%) and Fb (36%). The elastic loading caused a decrease over time within some of the gas exchange measures (VCO_2, Vt, RER, and $ETCO_2$), however, within this study, elastic loading in combination with positive pressure breathing do not appear to cause any lung inflammation or any changes in lung physiology that might compromise normal function. Even though previous research has shown elastic loading and positive pressure breathing, individually, have resulted in lung decrements and reduced function, but utilized together, such as the systems in place within high performance aircraft and it's pilot, it appears a synergistic relationship is made that likely reduces the inflation influence of the OBOGS but generally allows efficient gas exchange within these high performance pilots, during strenuous, G-force creating maneuvers.

6.0 LIMITATIONS AND NEXT STEPS

6.1 Study Aim 1

This study was performed at rest under very controlled conditions, compared to typical stressors of pilots (G forces, variable inspiratory resistive and threshold loads, altered inspired gases, restrictive – elastic loads, anti G maneuvers, higher metabolic demands). Therefore, the study needs to be viewed in this context. There may be a required synergy of stressors that result in greater susceptibility to the inspiratory loading. High oxygen may increase the degree of atelectasis, dry air and reduced inflation (not allowing reversal of atelectasis) may be important. However, one of the subjects did demonstrate a more significant transient decline in lung physiology although remained asymptomatic. This may suggest some degree of individual susceptibility to the environmental stressors such as diving induced lung injury or high-altitude

pulmonary edema where only 8-12% of subjects appear to be susceptible. Lastly, subjects in this study were exposed to inspiratory loading at a very slow frequency, by manipulating expiratory loads, increasing length of inspiratory cycles, and the variability in thresholds of inspiratory and expiratory loading we may be able to better simulate what pilots are experiencing. Moreover, the resting condition and low metabolic rates also allow a certain adaptive strategy by subjects to minimize any negative aspects of the perturbations.

6.2 Study Aim 2

Within this study, we chose to create elastic loads that reduced lung volumes by approximately 20% and positive pressures that were set to 15 and 20 cmH_2O under room air conditions and essentially resting, unstressed conditions. While the elastic load may in the flight setting be both a countermeasure and eliminate the ability to revere atelectic areas of the lungs, in this controlled setting it may have offset some of the negative aspects of mechanical ventilation associated with overinflation. Also, we did not have the dry air, high oxygen inspiration that may also contribute to atelectasis and work synergistically with mechanical ventilation to produce lung inflammation or increase airway reactivity. The resting condition and low metabolic rates also allow a certain adaptive strategy by subjects to minimize any negative aspects of the perturbations.

Thus, future studies should likely include combinations of stressors with and without elastic thoracic loading to determine if these are additive in their impact on lung function. This would include a similar study to the present project but with high inspired oxygen levels, varying inspired oxygen levels and using dry gases. In addition, it may be interesting to breathe this mixture for long periods of time off the ventilator to determine if this sets an individual up for atelectasis followed by periods of rapid mechanical ventilation which may result in regional hyperinflation or over stretch and inflammation or increased airway reactivity. Studies have

suggested that non reversed atelectasis through deep inhalations or sighs may over time be harder to reverse.

In addition, stimulation of metabolic rate reduces the ability of subjects to develop breathing strategies that minimize alterations in lung function and interspersing periods of normal breathing and positive pressure breathing – inspiratory vs expiratory loading, may be important. Often lung injury patterns occur due to rapid transitions in inspiratory pressure patterns (transpulmonary, etc.,) and varying across loads with high negative intrathoracic pressures and with pressure assist may be more likely to contribute to stretch and micro injury of lung parenchyma or alterations in fluid flux. Even interspersing Mueller maneuvers – breath hold type maneuvers, within the altering pressure swings may be an additive stressor as blood volumes shift (in operational conditions) centrally and peripherally. It would also be helpful to have greater guidance on the extent of load created by thoracic restriction or upper pressure garments to determine how closely our loads aligned with true conditions. Earlier studies have shown the use of a thoracic elastic load to caused significant decreases in Vt during exercise and a reduction of peak exercise capacity, however, the subjects within this study were in a seated position and inactive during the ventilator sessions. [12, 13]

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

DoD	Department of Defense
RBWG	Restrictive Breathing Working Group
OBOGS	On-board oxygen delivery system
O_2	oxygen
SD	standard deviation
BMI	body mass index
MIPS	mean inspiratory pressure
PFT	pulmonary function test
SVC	slow vital capacity
FVC	forced vital capacity
MVV	maximal voluntary ventilation
T_I/T_{TOT}	inspiratory time to total breath time
FOT	forced oscillation technique
R	resistance
X	reactance
VO_2	oxygen consumption
VCO_2	exhaled carbon dioxide
Vt	tidal volume
RR	respiratory rate
VE	minute ventilation
RER	respiratory exchange ratio
VE/VCO_2	ventilatory equivalent for carbon dioxide
$ETCO_2$	end tidal carbon dioxide
HR	heart rate
SpO_2	oxygen saturation
ExNO	exhaled nitric oxide
DLCO	lung diffusing capacity for carbon monoxide
DLNO	lung diffusion capacity for nitric oxide
Qc	pulmonary blood flow
IC	inspiratory capacity
FEV_1	forced expiratory volume in one second
FEF_{25-75}	forced expiratory flow between 25 and 75% of FVC
FEFmax	maximum forced expiratory flow
FIVC	forced inspiratory vital capacity
FEF_{50}/FIF_{50}	50% of forced expiratory flow to 50% of forced inspiratory flow ratio
FIFmax	
PTP	pressure-time product
Piavg	average inspiratory pressure
Peavg	average expiratory pressure
Ti	inspiratory time
Te	expiratory time
RV	residual volume

Fb	breathing frequency
ANOVA	analysis of variance
DM	surface area for gas exchange in the lungs
DM	surface area for gas exchange in the lungs
Q	cardiac output
DLCO/Q	alveolar capillary recruitment
DM/Vc	functional unit of diffusion
Δ	change score
Hz	Hertz