Diaphragm Force and Mitochondrial Function Following Enhanced Nitric Oxide Availability During Mechanical Ventilation

San Diego State University
School of Exercise & Nutritional Sciences

Simon P. Pierce, Jennah J.M. Brown, Daniel T. Cannon & Leonardo Nogueira

Laboratory of Muscle Contractile Function and Repair; School of Exercise and Nutritional Sciences; College of Health and Human Services; San Diego State University, San Diego, CA

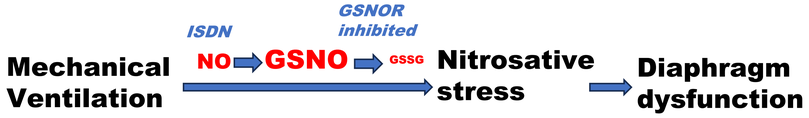
american physiology summit
APRIL 4–7, 2024
LONG BEACH

Introduction

During mechanical ventilation (MV), diaphragm force is impaired, affecting patient survival, especially during prolonged MV. This is known as ventilator-induced diaphragm dysfunction (VIDD), and VIDD may be accelerated by intramyofiber oxidative stress. During treatment of acute respiratory distress syndrome (ARDS), patients are treated with nitric oxide (NO) gas, and it can diffuse from lungs to diaphragm myofibers. NO in cells and muscle fibers reacts with the antioxidant glutathione (GSH), leading to the production of S-nitrosoglutathione (GSNO). GSNO accumulation accelerates the transfer of the nitroso group (-SNO) of GSNO to protein cysteines, a process named S-nitrosylation, which in muscle has been shown to reduce contractile and Ca^{2+} handling protein function. Our group has demonstrated that S-nitrosylation of muscle contractile proteins regulate myosin, muscle force, and myofiber fatigue resistance (Nogueira et al *BiochemJ* 2009; Figueiredo-Freitas et al. *AORS* 2015; Bailey et al *JPhysiol* 2019). Concentration of GSNO in cells is controlled by the enzyme GSNO reductase (GSNOR), which consumes GSNO. However, little is known whether increased NO availability or protein S-nitrosylation can affect diaphragm function during mechanical ventilation, accelerating or preventing VIDD. We proposed to investigate whether increasing NO availability with isosorbide dinitrate, a pharmacological NO-donor, or inhibiting S-nitrosoglutathione reductase (GSNORi) during MV could affect *ex vivo* diaphragm force and mitochondrial respiration.

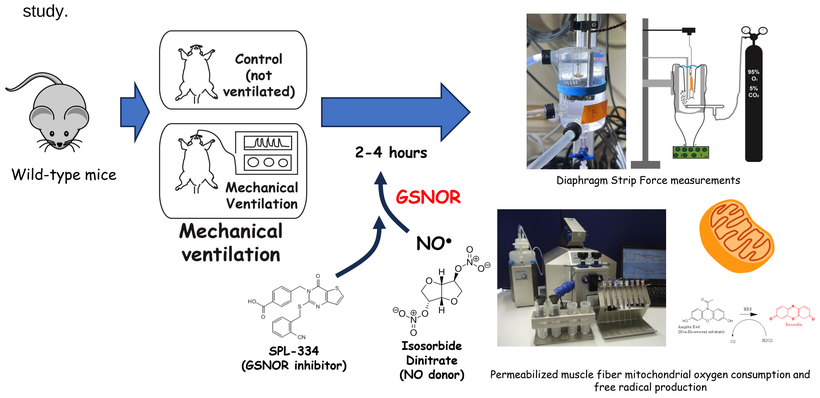
Hypothesis

We hypothesize that increase in intracellular concentration of GSNO in the diaphragm by inhibiting GSNO reductase (GSNOR) leads to a greater nitrosative stress in diaphragm myofibers under mechanical ventilation. This ultimately accelerates loss of diaphragm force, reduces myofiber mitochondrial oxygen consumption, and enhances ROS production.



Methods

All procedures were approved by SDSU-IACUC (protocol # 22-04-003N). 12-14 weeks old male C57BL/6J mice (Cat# 664) were obtained from Jackson Lab. A total of **107** mice were used in this study.



Mechanical Ventilation: Each mouse was tracheostomized and connected to a mechanical ventilator (Peak Inspiratory Pressure (PIP) ~ 10 cmH_2O, positive-end expiratory pressure (PEEP) ~ 3.5 cmH_2O, V_T 8 mL/kg, and breathing frequency 150/min, for 2 to 6 h. Experiments were interrupted if arterial oxygen saturation was below 90%.

Diaphragm Strip Force measurements: Diaphragm strips were subjected to 500 ms trains, 0.2 ms pulses, 0.6 A, 1-300 Hz contractions at 37ºC. After contractions, strip length was measured, blotted dry and weighed, and cross-sectional area (CSA) was determined by dividing the muscle mass with the product of muscle length and muscle density (1.06 mg/mm³) and reported as N/cm².

Diaphragm mitochondrial O_2 consumption and H_2O_2 production: Fluoro-respirometry of permeabilized fiber bundles were performed. Substrates provided were glutamate, malate, ADP, and succinate. Amplex Red used for H_2O_2 detection.

Results

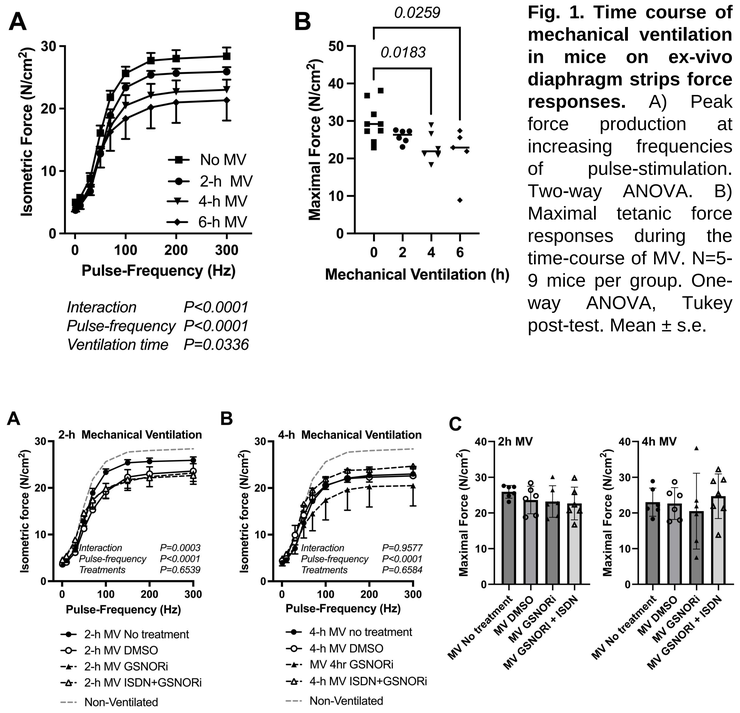


Fig. 1. Time course of mechanical ventilation in mice on ex-vivo diaphragm strips force responses. A) Peak force production at increasing frequencies of pulse-stimulation. Two-way ANOVA. B) Maximal tetanic force responses during the time-course of MV. N=5-9 mice per group. One-way ANOVA, Tukey post-test. Mean ± s.e.

Fig. 2. Effects of GSNORi, or GSNORi + ISDN, or vehicle (DMSO) on ex-vivo diaphragm strips force responses in mice ventilated for 2 (A) or 4 (B) hours. Data are shown as peak force production at increasing frequencies of pulse-stimulation. C) Maximal force. Two-way ANOVA. N=6-7 mice per group, Mean ± s.e.

Results

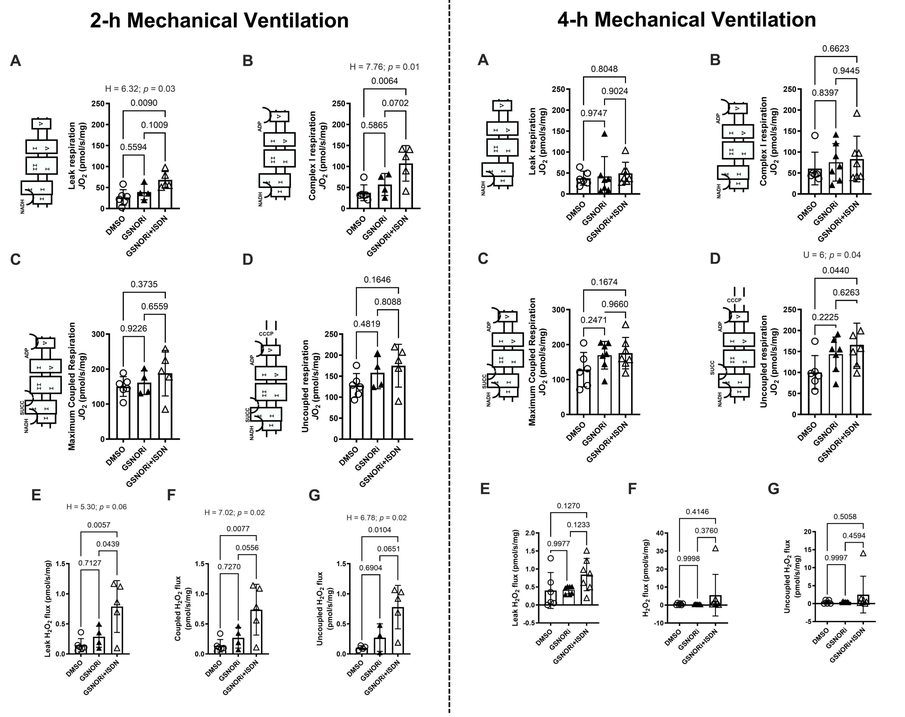


Fig. 3. Effects of GSNORi, or GSNORi + ISDN, or vehicle (DMSO) during 2h or 4h mechanical ventilation *in vivo* on permeabilized diaphragm myofiber oxygen consumption (A-D) and hydrogen peroxide production (E-F). N=6-7 mice per group, Mann-Whitney or Kruskall-Wallis statistics (top data), or Tukey test (connecting data), Mean ± s.d.

Acknowledgements

We would like to thank Dr. Leonardo Ferreira (Duke University School of Medicine) and Dr. Christopher Wilson (Loma Linda University) for thoughtful comments. Research is supported by the Tobacco-Related Disease Research Program Research Awards (T32IR5221 and T29KT0397CA to L.N.), SDSU SEED grant (to. L.N.), and SDSU-ENS Student Research Grant Award (To S.P.P).

TRDRP Tobacco-Related Disease Research Program
SDSU Seed Grant Program

Contact

spierce7234@sdsu.edu
lnogueira@sdsu.edu

Results Summary

- Ex-vivo force of diaphragm strips was statistically reduced starting at 4 hours of mechanical ventilation.
- Treating mice with GSNORi or ISDN + GSNORi did not affect ex-vivo force of diaphragm strips during mechanical ventilation.
- Treating mice with ISDN + GSNORi during mechanical ventilation for 2 hours produced increase in leak respiration and complex I respiration in permeabilized myofibers mitochondrial. However, after 4h of MV, increase was only present in uncoupled respiration.
- GSNORi + ISDN increased H_2O_2 production in myofibers after 2 hours of mechanical ventilation.
- Although increases in NO-dependent GSNO availability increased mitochondrial respiration and ROS production but did not accelerate VIDD during the short period of mechanical ventilation.