Received: 2 February 2021 | Revised: 14 April 2021 | Accepted: 16 April 2021

DOI: 10.1111/cts.13081

BRIEF REPORT

Hydrogen sulfide donor protects against mechanical ventilation-induced atrophy and contractile dysfunction in the rat diaphragm

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Funding information
This work was supported by JSPS KAKENHI Grant Number 15KK0131 and 19K22819 (N.I.-S.) and NIH R01, R01AR064189 (S.K.P.).

Abstract: Mechanical ventilation (MV) is a clinical tool providing adequate alveolar ventilation in patients that require respiratory support. Although a life-saving intervention for critically ill patients, prolonged MV results in the rapid development of inspiratory muscle weakness due to both diaphragmatic atrophy and contractile dysfunction; collectively known as “ventilator-induced diaphragm dysfunction” (VIDD). VIDD is a severe clinical problem because diaphragmatic weakness is a risk factor for difficulties in weaning patients from MV. Currently, no standard treatment to prevent VIDD exists. Nonetheless, growing evidence reveals that hydrogen sulfide (H_2S) possesses cytoprotective properties capable of protecting skeletal muscles against several hallmarks of VIDD, including oxidative damage, accelerated proteolysis, and mitochondrial damage. Therefore, we used an established animal model of MV to test the hypothesis that treatment with sodium sulfide (H_2S donor) will defend against VIDD. Our results confirm that sodium sulfide was sufficient to protect the diaphragm against both MV-induced fiber atrophy and contractile dysfunction. H_2S prevents MV-induced damage to diaphragmatic mitochondria as evidenced by protection against mitochondrial uncoupling. Moreover, treatment with sodium sulfide prevented the MV-induced activation of the proteases, calpain, and caspase-3 in the diaphragm. Taken together, these results support the hypothesis that treatment with a H_2S donor protects the diaphragm against VIDD. These outcomes provide the first evidence that H_2S has therapeutic potential to protect against MV-induced diaphragm weakness and to reduce difficulties in weaning patients from the ventilator.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Mechanical ventilation (MV) results in diaphragm atrophy and contractile dysfunction, known as ventilator-induced diaphragm dysfunction (VIDD). VIDD is important because diaphragm weakness is a risk factor for problems in weaning patients from MV. Currently, no accepted treatment exists to protect against VIDD. Growing evidence reveals that hydrogen sulfide (H_2S) donors protect skeletal muscle against ischemia-reperfusion-induced injury. Nonetheless, it is unknown if treatment with a H_2S donor can protect against VIDD.

WHAT QUESTION DID THIS STUDY ADDRESS?
Can treatment with an H_2S donor protect against VIDD?
WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
This study provides the first evidence that treatment with a H_2S donor protects against VIDD.
HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
These new findings provide the basis for further exploration of H_2S donors as a therapy to prevent VIDD and reduce the risk of problems in weaning patients from MV.

INTRODUCTION

Mechanical ventilation (MV) provides ventilatory support to patients that are incapable of producing adequate alveolar ventilation on their own. Worldwide, MV is used to support pulmonary gas exchange in 13–20 million patients annually.[1] Although MV is a life-saving intervention for many critically ill patients, a negative consequence of prolonged MV is the rapid development of diaphragm muscle weakness. This MV-induced diaphragmatic weakness occurs due to both muscle fiber atrophy and contractile dysfunction and this syndrome is termed "ventilator-induced diaphragm dysfunction" (VIDD).[2] VIDD is an important clinical problem because diaphragmatic weakness is a major risk factor for difficulty in "weaning" patients from MV.[3] The failure to wean extends time on the ventilator and markedly increases morbidity and mortality.[4] Currently, no standard therapy exists to prevent VIDD and, therefore, studies are needed to identify therapeutic agents capable of protecting the diaphragm against ventilator-induced weakness.

Multiple investigations have delineated the cellular events leading to VIDD and identified key biological targets for pharmacological intervention. In this regard, MV-induced diaphragmatic wasting occurs due to both a reduction in protein synthesis and accelerated proteolysis with proteolysis dominating during the first 12–18 h of MV.[5] Importantly, evidence reveals that MV-induced reactive oxygen species (ROS) generation is required to activate proteases and suppress protein synthesis in diaphragm fibers.[6–8] Although the MV-induced increase in ROS production in the diaphragm occurs at several locations within muscle fibers, mitochondrial ROS emission plays a dominant role in the development of VIDD.[7] Indeed, oxidative stress and damage to diaphragm mitochondria are hallmarks of VIDD.[7–9] Therefore, an efficacious therapy to protect against VIDD would likely protect against both MV-induced oxidative stress and mitochondrial damage.

Hydrogen sulfide (H_2S) is a water-soluble gas produced in humans and other mammals that can have both toxic and therapeutic effects.[10,11] Interestingly, at low (i.e., micromolar) concentrations, H_2S is not toxic and is cytoprotective against ischemia-reperfusion injury in both myotubes and skeletal muscles.[12–14] The specific mechanisms responsible for H_2S-facilitated protection against ischemia-reperfusion injury remains debatable but it appears that H_2S-mediated cytoprotection is multifactorial, including scavenging of ROS, increasing antioxidant enzyme expression, activating potassium ATP channels, and protecting mitochondria against injurious events.[15,16] These cytoprotective properties of H_2S suggest that this molecule has therapeutic potential to protect against VIDD; nonetheless, the proficiency of H_2S to prevent VIDD has not been investigated. Using an established preclinical model of MV, we tested the hypothesis that treatment with an H_2S donor (sodium sulfide) will protect against VIDD.

METHODS

Animals

Experimental protocols were approved by the University of Florida Institutional Animal Care and Use Committee. Female Sprague-Dawley rats (4 months old, ~280 g body weight) were selected for study because effects of prolonged MV on diaphragm fibers is identical in male and female rats,[9,17] and female body weights remain relatively stable from 3 to 8 months of age.

Experimental design

Animals were assigned to one of four experimental groups (*n* = 9–10/group): (1) 12 h of spontaneous breathing; treated with saline (SB-Sham); (2) 12 h of spontaneous breathing, treated with the H_2S donor, sodium sulfide (SB-H_2S); (3) 12 h of MV, treated with saline (MV-Sham); and (4) 12 h of MV; treated with sodium sulfide (MV-H_2S).

Mechanical ventilation protocol

Surgical procedures were performed using aseptic techniques. Complete details for the MV protocol have been provided previously.[7] Briefly, animals in the MV groups were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight). Upon reaching a surgical plane of anesthesia, animals received an intramuscular injection of glycopyrrolate (0.04 mg/kg body weight) and an intraperitoneal injection of sodium sulfide (50 μmol/kg body weight[18]) or saline. Animals were tracheostomized and ventilated with a pressure-controlled ventilator (Servo Ventilator 300; Siemens, Munich, Germany) utilizing the "controlled" mode for 12 h. Both the carotid artery and jugular vein were cannulated for measurement of arterial blood pressure and continuous infusion of anesthesia (sodium pentobarbital, ~10 mg/kg body weight/h), respectively. Arterial blood samples were obtained periodically and analyzed to determine arterial blood gas tension and pH (GEM Premier 3000; Instrumentation Laboratory).

During prolonged MV, continuous care was provided to the animals as described previously.[7] Animals were also given glycopyrrolate (0.04 mg/kg) intramuscularly every 2 h throughout MV to reduce airway secretions. Following 12 h of MV, the diaphragm was rapidly removed for subsequent analysis.

Spontaneous breathing protocol

Animals in the SB-Sham and SB-H_2S groups were anesthetized (sodium pentobarbital, 60 mg/kg body weight, intraperitoneal); animals then received an intraperitoneal injection of sodium sulfide (50 μmol/kg body weight) or saline. Animals breathed spontaneously for 12 h and received continuous care identical to the MV animals. Following 12 h of spontaneous breathing, the diaphragm was rapidly removed for subsequent analysis.

Diaphragmatic contractile properties

Contractile properties of diaphragm muscle strips were determined in vitro, as described previously.[7] Force production was normalized to muscle cross-sectional area (CSA).

Myofiber cross-sectional area

Cross-sections from frozen costal diaphragm muscle samples were cut at a thickness of 10 μm. Unfixed cryosections were stained for dystrophin (RB-9024-R7; Thermo Scientific), myosin heavy chain I (A4.840; Hybridoma Bank), and myosin heavy chain IIa (SC-71; Hybridoma Bank) for CSA analysis. CSA was analyzed with Scion Image software (National Institutes of health [NIH]).

Mitochondrial isolation and measurement of mitochondrial respiration

Mitochondria were isolated from diaphragm muscle and mitochondrial respiration was measured polarographically, as previously described.[7] Maximal adenosine diphosphate (ADP)-stimulated respiration (state 3) was obtained using 2 mM pyruvate and 2 mM malate in the presence of 0.25 mM ADP and state 4 respiration was recorded following the complete phosphorylation of ADP. The respiratory control ratio (RCR) was computed by dividing state 3 by state 4 respiration.

Western blot analysis

Western blots were performed as described previously.[19] Briefly, membranes were blocked in 5% milk solution, followed by incubation with primary antibodies: αII-spectrin (sc48382; Santa Cruz, Dallas, TX), 4-Hydroxynoneal (4-HNE; ab46545; Abcam, Cambridge, MA), superoxide dismutase 2 (SOD2; sc-30080; Santa Cruz), catalase (ab16731; Abcam), nuclear factor erythroid 2-related factor (Nrf2; sc-722; Santa Cruz), sirtuin-3 (Sirt3; 4904; Cell Signaling), cystathionine β-synthase (CBS; sc-133154; Santa Cruz), cystathionine γ-lyase (CSE; sc-374249; Santa Cruz), 3-mercaptopyruvate sulfurtransferase (3MST; sc-374326; Santa Cruz), and Cysteinyl-tRNA synthetase 2 (CARS2; HPA041776; Atlas Antibodies). For secondary incubation, membranes were exposed to either Alexa Fluor 680 IgG or 800 IgG (Thermo Scientific) for 1 h. Membranes were scanned and analyzed with the Li-Cor Odyssey Infrared Imager (Li-Cor Biosciences) using Odyssey 2.1 software. All westerns were normalized to total protein (Li-Cor Biosciences) or VDAC (sc-8829; Santa Cruz).

Statistical analysis

The sample size for experimental groups was selected following a power analysis. Comparisons between groups were made by one- or two-way analysis of variance (ANOVA) where appropriate. Planned comparisons were used appropriately. The p value less than 0.05 was established as the benchmark for statistical significance. Data are reported as mean values ± SD.

RESULTS

Systemic response to MV

No differences existed between experimental groups in animal body weight, heart rate, arterial blood gases, and arterial pH following the experimental protocol (Table S1).

H_2S donor protects against VIDD

To determine if an H_2S donor can protect the diaphragm against VIDD, we measured both diaphragm contractile properties and the CSA of diaphragm fibers. Our results confirm that the H_2S donor shielded the diaphragm against MV-induced contractile dysfunction at both submaximal and maximal stimulation frequencies and protected all fiber types against MV-induced atrophy (Figure 1a,b).

H_2S donor protects against VIDD by preventing MV-induced oxidative stress and protease activation in diaphragm fibers

To investigate the mechanisms responsible for the H_2S donor-mediated protection against VIDD we measured a biomarker of oxidative stress, mitochondrial respiration, and the activities of calpain and caspase-3 in the diaphragm. As expected, compared to SB animals, prolonged MV (MV-Sham animals) resulted in oxidative stress as evidenced by the increased abundance of 4-HNE conjugated proteins in the diaphragm. Notably, treatment with the H_2S donor protected diaphragm fibers against this MV-induced oxidative stress (Figure 2a). To determine if the H_2S donor protected diaphragm mitochondria from MV-induced uncoupling, we measured the RCR. As revealed by the significant decline in the RCR, prolonged MV promotes uncoupling of diaphragm mitochondria; importantly, this MV-induced decrease in mitochondrial uncoupling was absent in the H_2S donor-treated animals (Figure 2b). Finally, MV activated both calpain and caspase-3 proteases in the diaphragm of MV-Sham animals as evidenced by increased αII-spectrin specific degradation products for both calpain (145 kDa) and caspase-3 (120 kDa); notably, treatment with the H_2S-donor prevented the activation of these proteases (Figure 2c,d).

Impact of H_2S donor on diaphragm fiber capacity to generate H_2S

The endogenous production of H_2S in mammalian cells is regulated by several enzymes including[11]: CBS, CSE, and 3MST. To determine if an H_2S-donor influences the endogenous potential to generate H_2S in diaphragm fibers, we measured the abundance of CBS, CSE, and 3MST in the diaphragm. No differences existed between groups in the abundance of CSE and 3MST. In contrast, diaphragmatic levels of CBS were significantly higher in MV-H_2S animals compared to Sham animals (Figure S1).

Mechanisms responsible for H_2S donor-mediated protection against oxidative stress

H_2S can protect cells against oxidative damage by directly scavenge ROS and by activating cell signaling pathways that

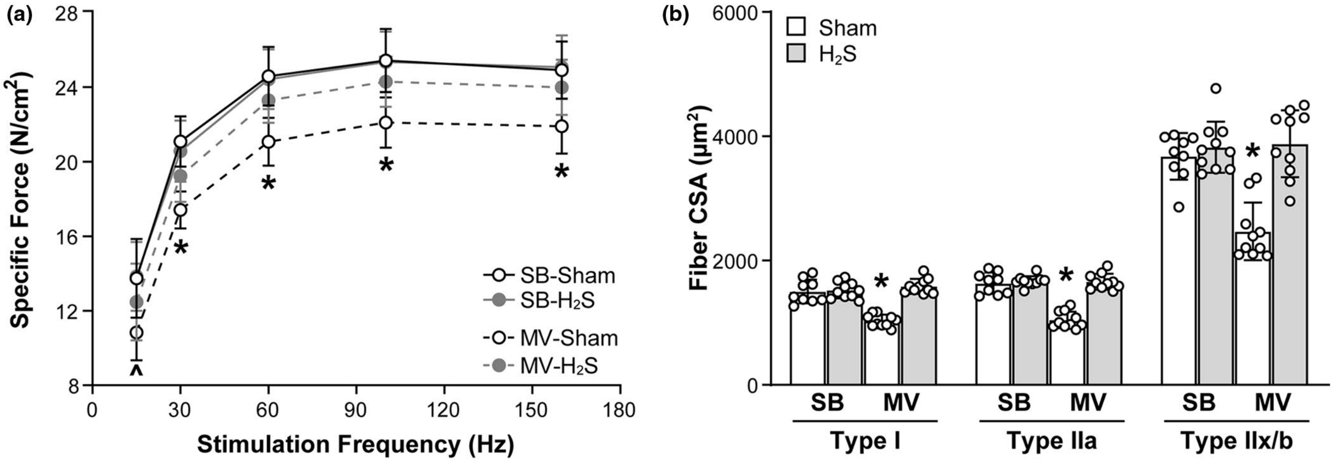

FIGURE 1 Hydrogen sulfide (H_2S) donor prevents diaphragm contractile dysfunction and fiber atrophy induced by mechanical ventilation. (a) Diaphragm specific force production as a function of the stimulation frequency (i.e., force-frequency curve) measured in vitro in costal diaphragm strips following 12 h of mechanical ventilation (MV) or spontaneous breathing (SB). (b) Diaphragm muscle fiber cross-sectional area in type I, type IIa, and type IIx/b fibers. Values are means ± SD. ^Significantly different versus SB-Sham and MV-Sham. *Significantly different versus all groups ($p < 0.05$)

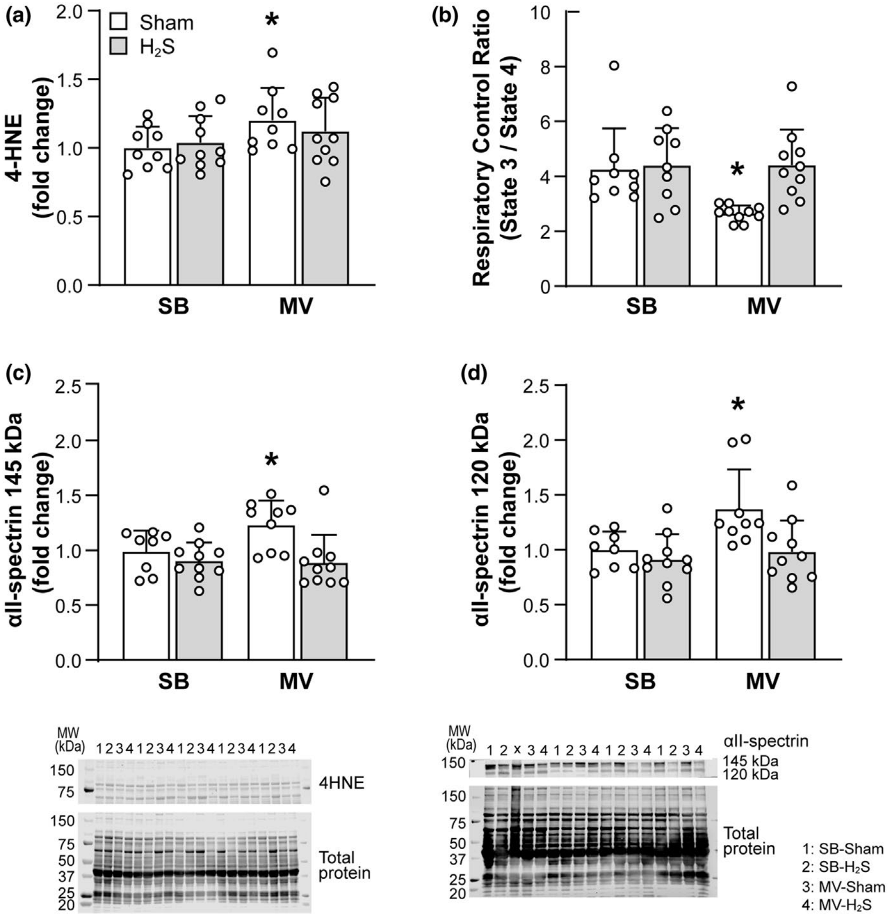

FIGURE 2 Hydrogen sulfide (H$_2$S) donor prevents mechanical ventilation-induced oxidative stress, mitochondrial dysfunction, and protease activation in diaphragm. (a) The relative abundance of 4-hydroxynonenal (4-HNE)-modified proteins (index of lipid peroxidation). (b) Mitochondrial respiratory control ratio (state 3/state 4; RCR). (c) Calpain-mediated cleavage of αII-spectrin releases a specific breakdown product at 145 kDa; abundance of this product is a surrogate biomarker of calpain activity. (d) Caspase-3 mediated cleavage of αII-spectrin releases a specific breakdown product at 120 kDa; abundance of this product is a surrogate biomarker of caspase-3 activity. Note that the lane labeled as "x" in the gel scan of αII-spectrin was excluded from the analysis. Values are means ± SD. *Significantly different versus all groups ($p < 0.05$)

promote the expression of antioxidant enzymes. Specifically, H$_2$S promotes the synthesis of cellular antioxidants by increased expression/activity of Nrf2 and Sirt3.[15] Our results are consistent with the concept that treatment with the H$_2$S donor protected against MV-induced oxidative stress, in part, by direct scavenging of ROS because no differences existed between groups in diaphragmatic levels of Nrf2 and Sirt3 (Figure S2).

Finally, because treatment of animals with the H$_2$S donor protected diaphragmatic mitochondria against MV-induced dysfunction, we determined the abundance of three key mitochondrial antioxidants; catalase, SOD2, and CARS2. No group differences existed in the abundance of catalase and SOD2 (Figure S3). However, compared to MV-Sham, mitochondrial levels of CARS2 were significantly higher in the MV-H$_2$S group (Figure S4).

DISCUSSION

Overview of major findings

Our results provide the first evidence that an H$_2$S donor protects the diaphragm against VIDD. A discussion of the potential mechanisms responsible for the H$_2$S donor-mediated protection against VIDD and the potential clinical application of H$_2$S donors follows.

Mechanisms responsible for H$_2$S donor-mediated protection against VIDD

The discovery that H$_2$S is produced in mammalian tissues by enzymatic and nonenzymatic pathways led to the understanding that, at low concentrations, H$_2$S is an important physiological signaling molecule that contributes to normal cellular function.[10] Moreover, preclinical experiments confirm that H$_2$S protects cells during several pathophysiological conditions (e.g., ischemia-reperfusion injury), displaying both anti-inflammatory and antioxidant properties.[20] This evidence and subsequent preliminary experiments formed the foundation for our hypothesis that an H$_2$S donor can protect the diaphragm against VIDD.

Our results suggest that H$_2$S donor-mediated protection against VIDD is linked to protection against MV-induced oxidative stress, mitochondrial dysfunction, and protease activation in diaphragm fibers. Indeed, protection against MV-induced oxidative stress has been shown to prevent the activation of calpain and caspase-3 and protect against VIDD.[7,8] Nonetheless, the precise mechanism(s) to explain why an H_2S donor protects against MV-induced oxidative stress is uncertain. In this regard, our results reveal that increased mitochondrial levels of catalase and SOD2 are not responsible for the H_2S donor-mediated protection against oxidative stress and therefore, it is feasible that direct scavenging of ROS by H_2S played a role in protection.[21] Moreover, it is feasible that increased mitochondrial levels of CARS2 also contributed to the H_2S donor-mediated protection against oxidative damage. Although it is unclear how the H_2S donor increases in mitochondrial levels of CARS2, CARS2-mediated synthesis of cysteine hydropersulfide (CysSSH) can produce H_2S in the mitochondria and participate in mitochondrial respiration.[22] Further, CysSSH is a nucleophile that can protect against oxidative stress in cells.[10,23] It is possible that exogenous H_2S donors supplemented the endogenous H_2S synthesis in diaphragm fibers during prolonged MV[24,25]; this is a testable hypothesis worthy of future study. Clearly, future studies should investigate the specific role that CysSSH and other sulfur species play in protecting against VIDD.

Summary and future directions

These experiments provide the first evidence that an H_2S donor can protect against VIDD. This important new finding provides the scientific basis for additional experiments to determine the optimal dose and timing of treatment with H_2S donors to protect against VIDD. Further, our results provide incentive for future experiments to investigate the therapeutic potential of CysSSH and other cysteine polysulfide species to protect against VIDD.

CONFLICT OF INTEREST

The authors declared no competing interests for this work.

AUTHOR CONTRIBUTIONS

N.I.-.S and S.K.P. wrote the manuscript. N.I.-S. and S.K.P. designed the research. N.I.-S., A.J.S., A.B.M., J.M.H., and A.M.H. performed the research. N.I.-S. analyzed the data.

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REFERENCES

1. Adhikari NK, Fowler RA, Bhagwanjee S, Rubenfeld GD. Critical care and the global burden of critical illness in adults. *Lancet*. 2010;376:1339-1346.
2. Vassilakopoulos T, Petrof BJ. Ventilator-induced diaphragmatic dysfunction. *Am J Respir Crit Care Med*. 2004;169:336-341.
3. Kim WY, Suh HJ, Hong SB, Koh Y, Lim CM. Diaphragm dysfunction assessed by ultrasonography: influence on weaning from mechanical ventilation. *Crit Care Med*. 2011;39:2627-2630.
4. Beduneau G, Pham T, Schortgen F, et al. Epidemiology of weaning outcome according to a new definition. The WIND study. *Am J Respir Crit Care Med*. 2017;195:772-783.
5. Powers SK, Wiggs MP, Sollanek KJ, Smuder AJ. Ventilator-induced diaphragm dysfunction: cause and effect. *Am J Physiol Regul Integr Comp Physiol*. 2013;305:R464-R477.
6. Hudson MB, Hudson MB, Bradley Nelson W, et al. Partial support ventilation and mitochondrial-targeted antioxidants protect against ventilator-induced decreases in diaphragm muscle protein synthesis. *PLoS One*. 2015;10:e0137693.
7. Powers SK, Hudson MB, Nelson WB, et al. Mitochondria-targeted antioxidants protect against mechanical ventilation-induced diaphragm weakness. *Crit Care Med*. 2011;39:1749-1759.
8. Whidden MA, Smuder AJ, Wu M, et al. Oxidative stress is required for mechanical ventilation-induced protease activation in the diaphragm. *J Appl Physiol*. 2010;1985(108):1376-1382.
9. Shanely RA, Zergeroglu MA, Lennon SL, et al. Mechanical ventilation-induced diaphragmatic atrophy is associated with oxidative injury and increased proteolytic activity. *Am J Respir Crit Care Med*. 2002;166:1369-1374.
10. Murphy B, Bhattacharya R, Mukherjee P. Hydrogen sulfide signaling in mitochondria and disease. *FASEB J*. 2019;33:13098-13125.
11. Paul BD, Snyder SH, Kashfi K. Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics. *Redox Biol*. 2020;38:101772.
12. Du JT, Li W, Yang J-Y, Tang C-S, Li QI, Jin H-F. Hydrogen sulfide is endogenously generated in rat skeletal muscle and exerts a protective effect against oxidative stress. *Chin Med J (Engl)*. 2013;126:930-936.
13. Henderson PW, Jimenez N, Ruffino J, et al. Therapeutic delivery of hydrogen sulfide for salvage of ischemic skeletal muscle after the onset of critical ischemia. *J Vasc Surg*. 2011;53:785-791.
14. Henderson PW, Singh SP, Weinstein AL, et al. Therapeutic metabolic inhibition: hydrogen sulfide significantly mitigates skeletal muscle ischemia reperfusion injury in vitro and in vivo. *Plast Reconstr Surg*. 2010;126:1890-1898.
15. Corsello T, Komaravelli N, Casola A. Role of hydrogen sulfide in NRF2- and sirtuin-dependent maintenance of cellular redox balance. *Antioxidants (Basel)*. 2018;7:129.
16. King AL, Lefer DJ. Cytoprotective actions of hydrogen sulfide in ischaemia-reperfusion injury. *Exp Physiol*. 2011;96:840-846.
17. Ichinoseki-Sekine N, Yoshihara T, Kakigi R, Sugiura T, Powers SK, Naito H. Heat stress protects against mechanical ventilation-induced diaphragmatic atrophy. *J Appl Physiol*. 2014;1985(117):518-524.
18. Wang MJ, Cai W-J, Li NA, et al. The hydrogen sulfide donor NaHS promotes angiogenesis in a rat model of hind limb ischemia. *Antioxid Redox Signal*. 2010;12:1065-1077.
19. Smuder AJ, Morton AB, Hall SE, et al. Effects of exercise preconditioning and HSP72 on diaphragm muscle function during mechanical ventilation. *J Cachexia Sarcopenia Muscle*. 2019;10(4):767-781.
20. Elrod JW, Calvert JW, Morrison J, et al. Hydrogen sulfide attenuates myocardial ischemia-reperfusion injury by preservation of mitochondrial function. *Proc Natl Acad Sci USA*. 2007;104:15560-15565.

21. Bhatia M. Hydrogen sulfide as a vasodilator. *IUBMB Life*. 2005;57:603-606.
22. Akaike T, Ida T, Wei F-Y, et al. Cysteinyl-tRNA synthetase governs cysteine polysulfidation and mitochondrial bioenergetics. *Nat Commun*. 2017;8:1177.
23. Sawa T, Motohashi H, Ihara H, Akaike T. Enzymatic regulation and biological functions of reactive cysteine persulfides and polysulfides. *Biomolecules*. 2020;10:1245.
24. Zhang HX, Jun-Ming DU, Ding Z-N, et al. Hydrogen sulfide prevents diaphragm weakness in cecal ligation puncture-induced sepsis by preservation of mitochondrial function. *Am J Transl Res*. 2017;9:3270-3281.
25. Yang R, Jia Q, Li Y, Mehmood S. Protective effect of exogenous hydrogen sulfide on diaphragm muscle fibrosis in streptozotocin-induced diabetic rats. *Exp Biol Med (Maywood)*. 2020;245:1280-1289.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Ichinoseki-Sekine N, Smuder AJ, Morton AB, Hinkley JM, Mor Huertas A, Powers SK. Hydrogen sulfide donor protects against mechanical ventilation-induced atrophy and contractile dysfunction in the rat diaphragm. *Clin Transl Sci*. 2021;14:2139–2145. https://doi.org/10.1111/cts.13081