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Neonatal rodent ventilation and clinical correlation in congenital diaphragmatic hernia

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Abstract

Introduction: Ventilator management is a critical part of managing congenital diaphragmatic hernia (CDH). We aimed to use a murine model and patient data to study CDH-associated differences in oxygenation, airway resistance, and pulmonary mechanics by disease severity.

Methods: We used the nitrofen model of CDH. For control and CDH rodents, data were collected within the first hour of life. Oxygen saturations (SpO_2) were collected using MouseOx, and large airway resistance and inspiratory capacities were collected using flexiVent. A single-center, retrospective review of term CDH infants from 2014 to 2020 was performed. Tidal volumes were collected every 6 h for the first 48 h of life or until the patient was taken off conventional ventilation. Newborns that were mechanically ventilated but had no pulmonary pathology were used as controls. CDH severity was defined using the CDH Study Group (CDHSG) classification system.

Results: Control rodents had a median SpO_2 of 94% (IQR: 88%–98%); CDH pups had a median SpO_2 of 27.9% (IQR: 22%–30%) ($p < 0.01$). CDH rodents had lower inspiratory capacity than controls (median: 110 µl, IQR: 70–170 vs. median: 267 µl, IQR: 216–352; $p < 0.01$). CDH infants had a lower initial SpO_2 than control infants. Overall, CDH infants had lower tidal volumes than control infants (median: 4.2 ml/kg, IQR: 3.3–5.0 vs. 5.4 ml/kg, IQR: 4.7–6.2; $p = 0.03$). Tidal volumes varied by CDHSG stage.

Conclusion: Newborns with CDH have lower SpO_2 and lower, CDHSG stage specific, tidal volumes than control infants. The nitrofen model of CDH reflects these differences. Rodent models may be useful in studying therapeutic ventilatory strategies for CDH infants.

KEYWORDS
congenital diaphragmatic hernia, hypoxia, neonatology, pulmonary function, ventilation

1 | INTRODUCTION

Congenital diaphragmatic hernia (CDH) affects 1 in 3000–5000 infants. CDH patient outcomes have improved over time, yet overall mortality remains roughly 30%.[1] CDH infants often suffer severe cardiopulmonary failure within hours of birth, and intubation and mechanical ventilation are critical components of their initial stabilization and ongoing management.[2,3] While there is widespread recognition of the importance of avoiding ventilator-induced lung injury and oxygen toxicity in CDH infants, data regarding optimal ventilatory parameters for this highly complex patient population are lacking.

Several studies exist regarding lung-protective ventilation, including the role of permissive hypercapnia, high-frequency oscillatory ventilation (HFOV), and limiting peak inspiratory pressures (PIP) when caring for CDH infants.[2,3] However, there is a broad range of described protocols and significant institutional variation in ventilation strategy, and uncertainty remains regarding how pulmonary mechanics differ not only between CDH and non-CDH infants, but also between CDH infants with low- versus high-risk disease.[4–6]

The objectives of this study were (1) to use a rodent model to understand CDH-associated changes in postnatal oxygenation and pulmonary volumes and resistance and (2) to compare these data to clinical data from newborn infants with CDH.

2 | METHODS

2.1 | Animal model of CDH

We used the well-established nitrofen-rodent model of CDH.[7] The animal protocol was approved by the Animal Welfare Committee (AWC-20-0047) within the McGovern Medical School at the University of Texas Health Science Center. Power analyses were performed to determine the optimal number of animal subjects required to observe significant differences under the proposed conditions (β = 0.20).

Time-pregnant Sprague-Dawley rats (ENVIGO) were fed 100 mg of Nitrofen (Sigma-Aldrich) in 1 ml of olive oil on gestational day (GD) 9.5 (±6 h). Dams in the control group were untreated. Pups were born on GD 22 and immediately taken for data collection. As previously reported, and in our own experience, roughly 70% of pups from the nitrofen-exposed dams have CDH.[7,8]

2.2 | Oxygenation and ventilation data

Oxygenation data were collected using the MouseOx® System (STARR Life Sciences Corp.). MouseOx® is a pulse oximeter designed to measure arterial oxygen saturation (SpO$_2$) in rodents.[9] Newborn pups were placed in the decubitus position on white 4 × 4 inch white gauze pads. The MouseOx® collar sensor was attached low around the pups' spine with the infrared sensors transilluminating the abdomen (Figure 1A). This configuration was chosen in collaboration with the MouseOx® team due to previous experience working with small rodents and because the thigh, foot, and throat sensors, all designed for adult rodents, were too large for newborn pups. Rodents and gauze were then loosely covered with an occlusive plastic covering to protect the sensors from extraneous light interference. Once connected to the sensor, oxygenation measurements were collected consecutively every minute (on the minute) for 10-min by a single, trained study team member.

Ventilation studies were performed using flexiVent (SCIREQ Inc.). FlexiVent is a murine ventilation system based on the linear single-frequency forced oscillation technique (FOT), as previously described.[10,11] This FOT system was used to determine inspiratory capacity (IC) and large airway resistance in CDH and control rodents. Newborn pups were anesthetized with inhalational isoflurane (4%; Clipper Distributing Company, LLC). When the pups were sufficiently anesthetized, they underwent surgical tracheostomy placement.

The tracheostomy was performed on a blue wax dissection tray on a heated surgical platform using either surgical loupes or a dissecting microscope. Pups were secured in the supine position with the neck and head in full extension, occasionally with a small piece of gauze used as a bump under the cervical spine to slightly hyperextend the neck. A transverse skin incision was made using dissection scissors, and the subcutaneous fat and muscle were bluntly separated (Figure 1B). The field was kept dry using cotton-tipped applicators and gauze. The proximal aspect of the trachea was carefully delivered, and dissection scissors were used to make a 1 mm incision in the anterior tracheal wall. FlexiVent Y-tubing, roughly 6 cm in length was pre-connected to a 21-gauge, 0.5 inch blunt-tipped catheter (Figure 1C). This catheter was gently introduced into the tracheal incision; placement was confirmed via direct visualization. The tracheostomy was secured using a drop of super glue followed by rubber cement (Figure 1D). Rodents were then moved, gently, to the flexiVent apparatus.

The flexiVent deep inflation maneuver, set at 30 cmH$_2$O, was used to obtain IC values, as previously described.[11] Serial deep inflation (recruitment) maneuvers were performed to gradually recruit compressed airway spaces in the newborn rodent lungs (Video S1). These recruitment breaths were continued until the IC values plateaued and began to stabilize or fall. The optimal IC value was recorded for pups in each group. We collected Newtonian resistance (Rn) values using the constant phase model of flexiVent. Rn represents the resistance of the central airways (trachea, mainstem bronchi). After completion of oxygenation and ventilation experiments, presence of CDH was confirmed by thoracotomy and direct examination of the diaphragmatic defect with herniated abdominal viscera.

2.3 | Clinical data

To understand clinical differences in oxygenation and ventilatory parameters between newborns with and without CDH, we

performed a single-center, retrospective review of term (gestational age ≥ 36 weeks) CDH infants at our tertiary children's hospital. The study period ran from 2018 through 2020. This study was approved by the Children's Memorial Hermann and McGovern Medical School Institutional Review Board (HSC-MS-21-0055). Newborns that were mechanically ventilated but had no primary pulmonary pathology (e.g., infants with tracheoesophageal fistula after surgical repair) were used as the control group. Exhaled tidal volumes (ml), displayed by the ventilator and recorded in the medical record, were collected from the first hour of life and every 6 h thereafter for the first 48 h of life or until the patient was taken off conventional ventilation (transitioned to extracorporeal life support [ECLS], HFOV, or taken to the operating room). These tidal volumes are reflective of a standardized CPG where PIP and positive end-expiratory pressures (PEEP) are optimized and limited (range: PIP: 20–26 cmH_2O; PEEP: 4–7 cmH_2O). The median tidal volume for each patient was used for analyses. Additionally, birthweight, first recorded SpO_2, and disease severity were also collected. Disease severity was defined using the CDH Study Group (CDHSG) classification system.[12] Stages A and B defects are considered low-risk, and Stages C and D or non-repaired infants are considered high-risk.

2.4 | Statistical analysis

Continuous data from the rodent ventilation studies were analyzed using t-tests, and clinical data were analyzed using the Mann–Whitney U-test for non-parametric data. For both the experimental and clinical data, Kruskal–Wallis tests followed by Dunn-test correction were used to adjust for multiple comparisons. Level of significance was set to $p < 0.05$ for all analyses. Data were analyzed using Stata/IC version 16.1 (StataCorp LP) and Prism 9 (GraphPad Software).

3 | RESULTS

3.1 | Rodent oxygenation and ventilation

To understand differences in immediate post-natal oxygenation between CDH and control rodents, a MouseOx® throat sensor was connected to the torso of CDH and control pups. We found the cervical and foot sensors designed for adult rodents to be too big for reliable neonatal measurements. Because CDH rodents universally die within 30–60 min of birth, SpO_2 values were collected every minute for the first 10 min of life. The CDH group ($n = 10$) included six
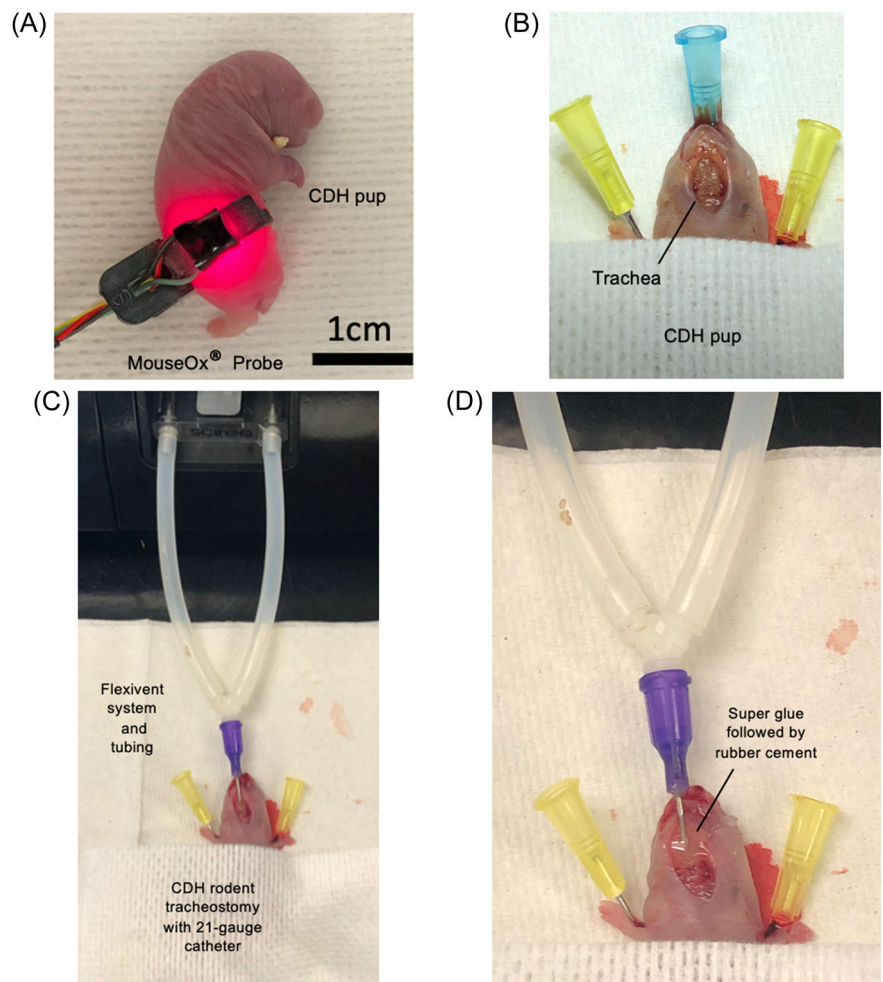
FIGURE 1 Rodent oxygenation and ventilation models. (A) MouseOx® probe connected around a congenital diaphragmatic hernia (CDH) pup in the decubitus position and placed on white gauze. (B) Representative image of tracheal dissection for newborn pup tracheostomy. (C) FlexiVent Y-shaped tubing and 21-gauge, 0.5-inch catheter tracheostomy. (D) Tracheostomy secured with super glue followed by rubber cement. [Color figure can be viewed at wileyonlinelibrary.com]

rodents with left-sided high-risk defects (C and D), two left-sided Stage B defect, and two right-sided high-risk defects. Control rodents ($n = 10$) had a median SpO_2 of 94% (IQR: 88%–97%), significantly higher than the SpO_2 of CDH rodents (median: 28%; IQR: 22%–30%) ($p < 0.001$). The median SpO_2 of pups exposed to nitrofen but without a diaphragmatic defect (CDH) ($n = 9$) was not different than control rodents, but was significantly higher than rodents with CDH ($p = 0.02$) (Figure 2A).

3.2 | Ventilation and pulmonary mechanics data

To analyze differences in CDH versus control pulmonary mechanics, rodent ventilation studies were performed using flexiVent. Newborn rodents underwent tracheostomy placement as described above. All CDH-group flexiVent data were obtained from pups with high-risk defects (Stages C and D). We found that large airway resistance

(trachea, mainstem bronchi) was similar between control and CDH rodents (Figure 2B). However, the IC of CDH rodents was nearly 60% lower than controls ($p < 0.001$) (Figure 2C).

3.3 | Clinical data

For the clinical analyses, a total of 47 CDH and 10 non-CDH neonates were identified. Of the CDH patients, seven were born at less than 36 weeks gestation, two patients presented after their initial resuscitation, four were initiated on HFOV or ECLS within 6 h of birth, and four were either not intubated or only intubated before going to the operating room, leaving a final cohort of 30 CDH and 10 non-CDH neonates who met prespecified inclusion criteria. Fourteen (46.7%) patients were CDHSG Stage A/B (4 A, 10 B), thirteen (43.3%) were CDHSG Stage C/D (9 C, 4 D), and three patients did not undergo CDH repair. Twenty-three (77%) of the
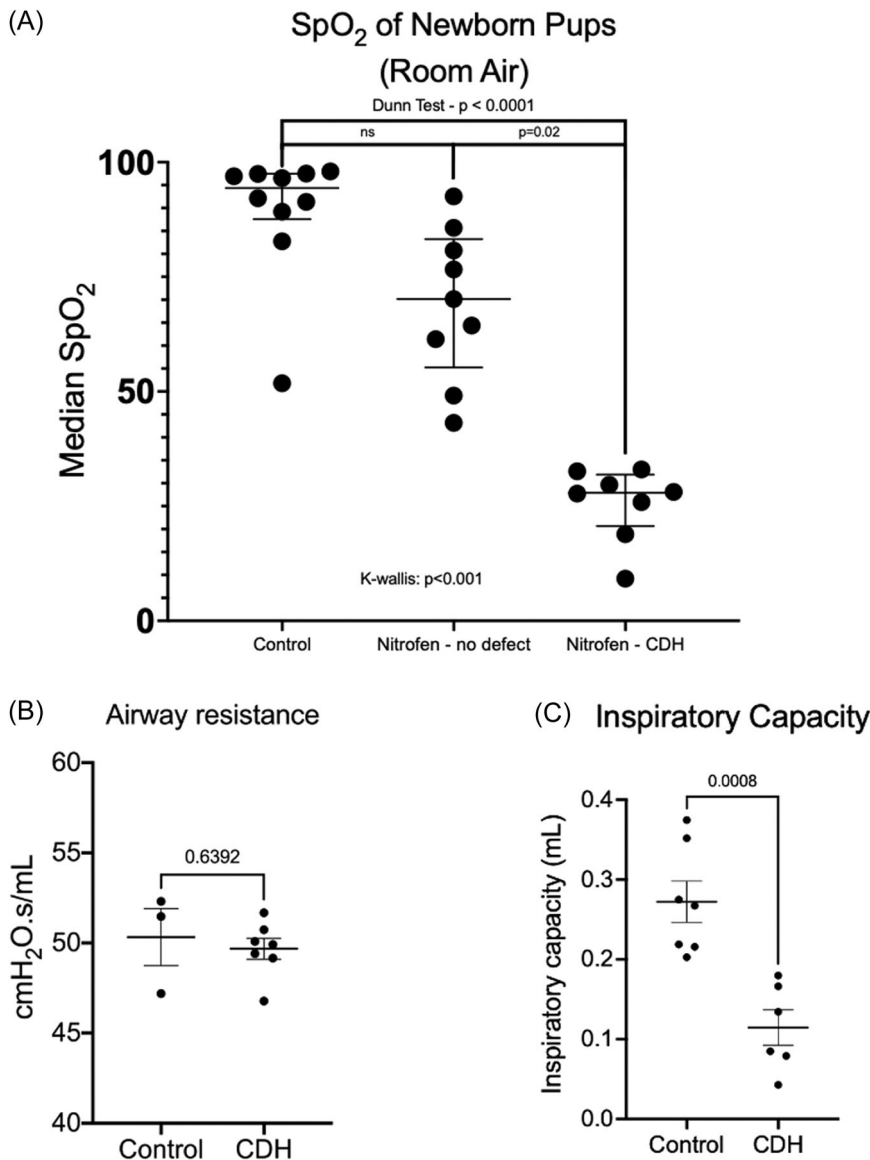
FIGURE 2 Rodent model pulmonary mechanics. (A) MouseOx® data for oxygen saturation (SpO_2) on room air for the first 10 min of life (congenital diaphragmatic hernia [CDH] and control, $n = 10$; Nitrofen—no defect, $n = 9$). ns, not significant. (B) Newtonian resistance (large airway resistance) values for control ($n = 3$) versus CDH ($n = 7$) rodents using flexiVent. t-test $p = 0.6932$. (C) Inspiratory capacity for control ($n = 7$) versus CDH rodents ($n = 6$) using flexiVent. t-test $p = 0.008$.

included CDH infants and all control infants survived to hospital discharge. CDH infants had a lower SpO_2 at birth than control infants ($p = 0.03$) (Figure 3A). Overall, CDH infants had lower tidal volumes than control infants (median: 4.2 ml/kg, IQR: 3.3–5.0 vs. 5.4 ml/kg, IQR: 4.7–6.2; $p = 0.03$). When risk-stratified, there was no difference in tidal volume between controls and low-risk CDH patients, but high-risk CDH infants had significantly lower tidal volumes than controls ($p = 0.02$) (Figure 3B). Patients with diaphragmatic agenesis (CDHSG Stage D) had a median exhaled tidal volume of 2.9 ml/kg, the lowest of all groups (Figure 4).

4 | DISCUSSION

Caring for infants with CDH requires nuanced ventilatory strategy and a robust, multidisciplinary team. Over the last several years, the importance of avoiding ventilator-induced lung injury in CDH infants has been increasingly recognized, but data regarding specific CDH-associated alterations in pulmonary mechanics are lacking.[2,3] We found that, in a rodent model of CDH, large airway resistance between CDH and control rodents is similar, but SpO_2 and IC are significantly decreased in CDH. Clinical data supported these findings, newborns with CDH, especially those with high-risk defects, have lower initial SpO_2 and have lower tidal volumes than neonates without pulmonary pathology.

Using the MouseOx® system, we found that nitrofen-exposed pups with diaphragmatic defects have a lower arterial SpO_2 in the immediate postnatal period than both control pups and nitrofen-exposed pups without CDH. These results are corroborated by our clinical data, in which CDH infants had a lower first SpO_2 than infants in the control group. Interestingly, rodents in the nitrofen-exposed group without CDH had slightly lower SpO_2 than control rodents,

though this did not reach statistical significance. This finding reinforces the two hit hypothesis in CDH and the idea that the physical herniation of abdominal organs into the chest in utero plays an important role in the pulmonary pathophysiology of CDH, including early postnatal hypoxia.[13] While mechanical compression resulting from organ herniation compromises organ development, an earlier, embryopathy at the subcellular, cellular and tissue levels of
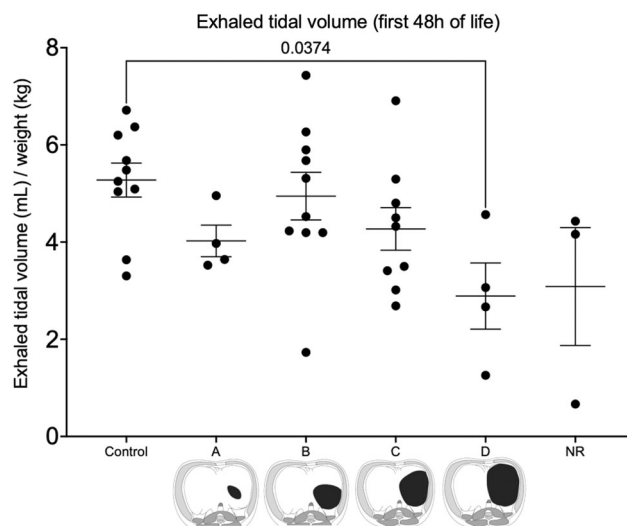
FIGURE 4 Median exhaled tidal volumes in the first 48 h of life or until taken off conventional ventilation based on congenital diaphragmatic hernia Study Group (CDHSG) stage. There is a statistically significant decrease in exhaled tidal volume from control infants to infants with diaphragmatic agenesis (CDHSG Stage D) (Dunn test, $p = 0.0374$). Kruskal–Wallis, $p = 0.04$. (Representative images used with permission from Lally et al. doi:10.1016/j.jpedsurg.2013.08.014).
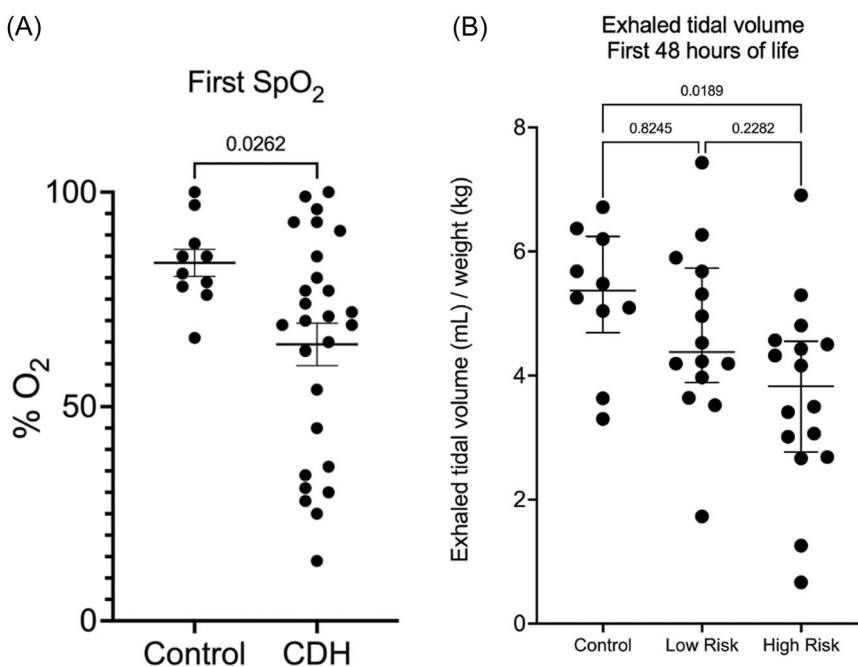
FIGURE 3 Clinical data. (A) First arterial oxygen saturation (SpO_2) recorded for newborn control infants versus those with congenital diaphragmatic hernia (CDH). Mann–Whitney U, $p = 0.0262$. (B) Exhaled tidal volumes (ml) per kilogram of bodyweight in the first 48 h of life or until taken off conventional ventilation based on risk stratification—controls, low-risk (CDH Study Group [CDHSG] Stage A/B), high-risk (CDHSG Stage C/D) and non-repaired infants. Kruskal–Wallis, $p = 0.02$.

the pulmonary vasculature, parenchyma, and ventricles represents the other hit. Our evidence suggests that nitrofen also replicates that nonmechanical component of the two-hit hypothesis in rodents. Prior investigation has found nitrofen-exposed rodents to have disrupted pulmonary development (GDs: 11–12) even before diaphragm formation/closure (~GD 16).[14,15] While the present work focuses on functional rather than histologic outcomes, nitrofen-associated changes in the pulmonary parenchyma and vasculature represents an important area of future study. Postnatal hypoxia in CDH infants is well described and is attributable to pulmonary hypoplasia and pulmonary hypertension.[16] While our results may be unsurprising, we report a novel application of the MouseOx® system to the rodent model of CDH. Other methods of studying oxygenation in newborn pups such as histologic staining or blood gas measurements are more cumbersome, less practical, and less clinically applicable. This study represents a quantifiable, reproducible way to study oxygenation in living CDH rodents and sets the stage for future works studying the effect of changes in ventilation strategy and/or novel therapies.

In studying rodent pulmonary mechanics with flexiVent, we found that CDH and control rodents have similar large airway resistances but significantly different inspiratory capacities. While limited series describe tracheal malformations in CDH patients, CDH is a primarily pulmonary parenchymal and pulmonary vascular pathology.[17,18] Our findings suggest that obstructive large airway patterns are not a critical component of the pathophysiology of CDH and corroborate existing literature that suggests that rodents with restrictive pulmonary pathology have similar large airway resistance values compared to controls.[19] Using flexiVent, we found that the IC of CDH rodents was 40%–60% that of control rodents. Our findings show a slightly larger difference in lung capacity than other existing reports. Gallindo et al.[20] estimated that rodents with CDH should be ventilated at a tidal volume that was 67% that of control rodents, but their data were based on estimates of ideal tidal volumes based on visualization of successful chest rise. Flemmer et al.[21] used FOT and found that CDH rabbits had inspiratory capacities roughly 70% that of control rabbits. Our more extreme results could be due to our use of the FOT method in rodents and our large percent of high-risk defects.

Clinical data from infants with CDH supported our findings regarding decreased lung volumes.[22,23] We found that, in the first 48 h of life, while on conventional ventilation, CDH infants have significantly decreased tidal volumes than control infants. These differences were most apparent in high-risk defects—CDHSG Stage C or D—or infants who did not survive to diaphragm repair. This analysis adds to the body of existing literature regarding ventilator management of CDH infants.[5] CDH infants likely benefit from lower lung volumes to avoid iatrogenic lung injury. However, special attention may need to be given to defect severity early in the postnatal period, as our combined rodent and clinical data (taken together) suggest that patients with CDH have lung volumes of approximately 40%–80% when compared to controls. Our preliminary findings indicate that patients with high-risk CDH may benefit from pulmonary tidal volumes of 3–4 ml/kg, whereas low-risk

patients may tolerate slightly higher volumes of 4–5 ml/kg. More specifically, optimal tidal volume ranges (given our clinical practice guidelines), based on these data, stratified by CDHSG stage, are 4 ± 0.5 (A), 5 ± 1.0 (B), 4 ± 1.0 (C), and 3 ± 0.5 (D) ml/kg (control: 5.5 ± 1.0 ml/kg). Ventilating high-risk CDH patients at equal or higher volumes to low-risk or control infants may risk ventilator-associated lung injury. Traditionally, defect size is determined at the time of operative repair upon direct inspection of the diaphragm.[12] Emerging prenatal imaging data may be useful in risk stratifying infants before delivery in a way that could allow the medical team to optimize immediate postnatal care.

There are key limitations to this study. Both MouseOx® and flexiVent are designed for and primarily utilized in adult animal models, and we were not able to obtain neonatal data for all parameters (compliance, volume-pressure loops). Our method of collecting SpO_2 values, while valid and reproducible, is not previously reported elsewhere. CDH rodents universally die within 30–60 min of birth, so longer term experiments (hours to days of life) are not currently feasible. We performed pilot experiments with alternative ventilation equipment including the Harvard microVent (Harvard Apparatus) and the AVEA Ventilation System (Vyaire Medical), but neither was able to provide consistent pulmonary mechanic data for CDH rodents. However, based on our flexiVent data, our lab is currently exploring the pulmonary inflammatory effects of volume-control ventilation using the microVent system. Our clinical data are based on a limited number of patients from a single-center, so the results may not be broadly applicable to all CDH infants. Importantly, this study sets the foundation for future multicenter investigation into nuanced ventilatory management for CDH infants with different disease severity.

Despite these limitations, this study has several important takeaways. MouseOx® can be used in animal models of CDH to test treatment response to medical therapy of changes in ventilation. Our lab is collaborating with industry partners to test the effect of oxygen-carrying nanoparticles and hypoxia pathway modulators on the SpO_2 of CDH rodents. For our team and in the existing literature, neonatal rodent tracheostomy had a steep learning curve, but can be achieved reliably and reproducibly.[20] FlexiVent and other rodent ventilation systems can record a variety of measurements using different ventilation parameters. Whereas most of the limited CDH ventilation research has been done on the surgical rabbit model, this study lays the foundation for future investigation using the nitrofen model of CDH—which more closely resembles severe human CDH pathophysiology.[7,21] These techniques also give the opportunity to assess prenatal therapies that are administered in utero.

In conclusion, in a translational, rodent model of CDH, CDH rodents had lower SpO_2 values and lower inspiratory capacities than control rodents in the initial postnatal period. Similar changes were seen in neonates with CDH in the first 48 h of life, and larger defect size was associated with worse pulmonary mechanics. Oxygenation and lung-protective ventilation are critical components of CDH care, and the data and methods described in this study set the stage for ongoing exploration into hypoxia, pulmonary function, optimal

ventilator management tailored to disease severity, and targeted therapies for CDH.

AUTHOR CONTRIBUTIONS

Vikas S. Gupta: conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); project administration (equal); visualization (equal); writing—original draft (lead); writing—review & editing (equal). **Cory Wilson**: data curation (equal); investigation (equal); methodology (equal); resources (equal); software (equal); supervision (equal). **Elizabeth C. Popp**: data curation (lead); investigation (equal); writing—review & editing (equal). **Siqin Zhaorigetu**: data curation (equal); investigation (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); visualization (equal); writing—review & editing (equal). **Di Jin**: data curation (supporting); investigation (supporting); methodology (supporting); project administration (supporting); resources (supporting); writing—review & editing (supporting). **Amir Khan**: conceptualization (equal); methodology (equal); supervision (equal); validation (equal); writing—review & editing (equal). **Harry Karmouty-Quintana**: conceptualization (equal); project administration (equal); resources (equal); software (equal); supervision (equal); writing—review & editing (equal). **Kevin Lally**: conceptualization (equal); funding acquisition (equal); project administration (equal); resources (equal); supervision (equal); validation (equal); writing—review & editing (equal). **Scott Collum**: data curation (equal); project administration (equal); resources (equal); software (equal); supervision (equal). **Matthew Harting**: conceptualization (lead); funding acquisition (lead); project administration (lead); resources (lead); supervision (lead); validation (equal); writing—review & editing (equal).

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.