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Response to Resistive Loading in the Newborn Piglet

DENNIS E. MAYOCK, RICHARD J. BADURA, JON F. WATCHKO, THOMAS A. STANDAERT, AND DAVID E. WOODRUM

Division of Neonatal and Respiratory Diseases, Department of Pediatrics, University of Washington School of Medicine, Seattle, Washington 98195

ABSTRACT. The diaphragmatic force generation and electromyographic response to long-term (1 h) inspiratory resistive loading was examined in the newborn piglet during the 3rd postnatal wk of life. Minute ventilation decreased to approximately 50% of baseline level within 5 min of imposition of a severe resistive load and remained at this level for the duration of loading. The decrease in ventilation was secondary to a fall in tidal volume at a constant frequency. There was a significant increase in central nervous system output to the diaphragm as manifested by integrated diaphragmatic electromyogram. Progressive augmentation of this index of central drive continued throughout the period of loading. Functional residual capacity fell significantly by 60 min of inspiratory resistive loading. This strategy should allow greater force generation by placing the diaphragm at a more optimal length-tension relationship. However, the force generating capability of the diaphragm was compromised as assessed by force-frequency curve analysis. These results suggest that the diaphragm of the neonatal piglet fatigues during prolonged inspiratory resistive loading. (*Pediatr Res* 21: 121–125, 1987)

Abbreviations

IRL, inspiratory resistive loading
FRC, functional residual capacity
EMG, electromyogram
di, diaphragmatic
OD, outer diameter
Hz, Hertz of stimulation
V_T, tidal volume
Pga, gastric pressure
Pes, esophageal pressure
Pdi, transdiaphragmatic pressure
T_i, inspiratory time
T_{TOT}, respiratory cycle deviation
V_E, expired minute ventilation

Ventilatory failure, associated with parenchymal lung disease, is a common problem in neonatal medicine. A clear understanding of the mechanisms that result in ventilatory failure in the newborn is lacking. Recent studies involving adult human and animal subjects indicate that diaphragmatic fatigue may be an important cause of ventilatory failure. Bellemare and Grassino (1) found that the force reserve of the diaphragm was small in patients with chronic obstructive pulmonary disease and that this reserve could be rapidly exhausted by minor modifications in the breathing pattern. Furthermore, in normal adult volunteers and animals, the imposition of a large inspiratory flow resistive load results in mechanical failure of the diaphragm (2–13). These studies suggest that the diaphragm, the major force generating muscle of respiration, may fail to develop adequate tension following the imposition of an added respiratory load resulting, at times, in ventilatory failure.

Little is known about the ventilatory response of the newborn to altered respiratory mechanics. Abbasi *et al.* (14) studied the ventilatory response to short-term (10 min) inspiratory resistive loading in premature infants and found an immediate decrease in minute ventilation and tidal volume to 50% of control values. However, the infants were able to compensate for the added inspiratory resistance without significant hypoxia or hypercapnia. In the newborn monkey, respiratory compensation to short-term added inspiratory resistive loads is a function of postnatal maturation with older animals demonstrating the ability to defend minute ventilation whereas the young monkey had a significant fall in ventilation (15). The response of the neonate to long-term resistive loading has not been documented.

The current work was designed to evaluate the ventilatory response of the newborn piglet to a long-term (1 h) IRL, testing the hypothesis that failure to maintain baseline ventilation during inspiratory loading would occur secondary to fatigue of the diaphragm. Diaphragmatic fatigue was defined as a decrease in the force-frequency curve of the muscle (2, 16, 17).

METHODS

Five piglets, 14–21 days postnatal age, weighing 2.45–3.06 kg were studied. Only healthy animals with a respiratory rate of 15–30 breaths per minute, a PaO_2 of greater than 60 torr in room air, and a $PaCO_2$ equal to or less than 50 torr were accepted for study. The animals were anesthetized with an intravenous combination of chloralose (50 mg/kg) and urethane (200 mg/kg) and studied in the supine position. Subsequent infusions of anesthetic were utilized if the piglet developed jaw clonus. The trachea was surgically exposed, a metal tracheostomy tube (6.35 mm OD) was inserted in the distal trachea and connected to a Hans-Rudolph miniature one-way nonrebreathing valve (no. 2384, dead space-1.45 ml, inspiratory and expiratory resistance-0.01 cm H_2O/ml/s, measured at 600 mL/min). Expiratory flow was detected by a hot wire anemometer and the area under the flow trace integrated by a signal conditioner (Hewlett Packard 8815A) to determine V_T. A femoral artery and vein were cannulated to monitor blood gases and infuse anesthetic. A Corning 168 blood gas analyzer was utilized to determine arterial pH and blood gas tensions. Rectal temperature was continuously monitored and maintained between 38.5–39.5° C by a radiant warmer (18).

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Correspondence Dennis E. Mayock, M.D., Department of Pediatrics RD-20, University of Washington, Seattle, WA 98195.

The EMGdi was recorded differentially to ground from two multistranded steel wire electrodes placed approximately 1 cm apart into the paratendinous part of the right costal diaphragm via a subcostal extraperitoneal incision. Following electrode placement, the surgical incision was closed. The EMGdi signal was amplified, rectified, filtered from 30–3000 Hz, and integrated through a first order R-C network (time constant = 100 ms). Peak integrated (EMGdi) and slope moving average (EMGdi/0.1–0.4 s) of the EMGdi were determine for each animal during baseline and loaded conditions and were used to index central neural drive (19).

Thin walled latex balloons (4.5 cm long, Youngs Rubber Corp., Trenton, NJ) containing 0.5 ml of air were connected to polyethylene catheters (1.65 mm ID) and placed in the stomach and midesophagus to measure Pga and Pes, respectively. The catheters were connected to a differential pressure transducer to determine Pdi: Pdi = Pga–Pes.

Phrenic nerve stimulation was accomplished transvenously by catheter electrodes inserted into both external jugular veins (20–22). The electrode tips were positioned by initiating electrical signals from a nerve stimulator (Grass S48 Stimulator, Grass Medical Instruments, Quincy, MA) and advancing the catheters until the diaphragm contracted. Electrode position was further adjusted to produce a maximal synchronous contraction of both diaphragmatic leaves (22). Maximal response was judged by the size of the Pdi generated during tracheal occlusion at 100 Hz stimulation. Voltage was then increased 20% to ensure a "supramaximal" stimulus. The Pdi generated with the trachea occluded at end-expiration at different frequencies of phrenic nerve stimulation was utilized as an index of diaphragmatic force output to construct force-frequency curves. Pulses were of 0.2 ms duration and stimulation trains were applied for 2 s at 10, 20, 30, 50, 70, and 100 Hz. Changes in end-expiratory lung volume were assessed in three piglets during baseline and after 60 min of inspiratory loading (23). A schematic representation of the experimental model is shown in Figure 1.

Experimental procedures. Following animal preparation, baseline measurements including ventilatory parameters (respiratory frequency, V_T, T_i, spontaneously generated Pdi, pH, end-expiratory lung volume (n = 3), arterial blood gases, integrated EMGdi, and force-frequency curves were obtained. All animals breathed 50% O_2 during the study. A resistive load consisting of an 18-gauge stainless steel tube (average resistance R = 0.65 cm H_20/ml/s measured at 600 ml/min flow) was then added to the inspiratory port of the nonrebreathing valve. Measurement of ventilatory parameters, pH, and arterial blood gases were repeated at 5 and 60 min of loading breathing. Force-frequency curves and end-expiratory lung volumes measurements (n = 3) were repeated at 60 min.

Statistics. Each measurement of respiration (V_T, T_i, T_{TOT}, V_E), EMGdi and spontaneously generated Pdi consisted of the average of five breaths and is expressed as the mean for the five animals. Statistical analysis included the Student's two-tailed t test for paired data (Minitab Release 80.1, Penn State University, 1980) with correction for repeated measures. Analysis of variance and covariance with repeated measures (BMDP2V, University of California, Los Angeles, Los Angeles, CA 90024, rev 1984) was utilized to evaluate changes in the force-frequency curves. Significant differences were assumed if $p < 0.05$.

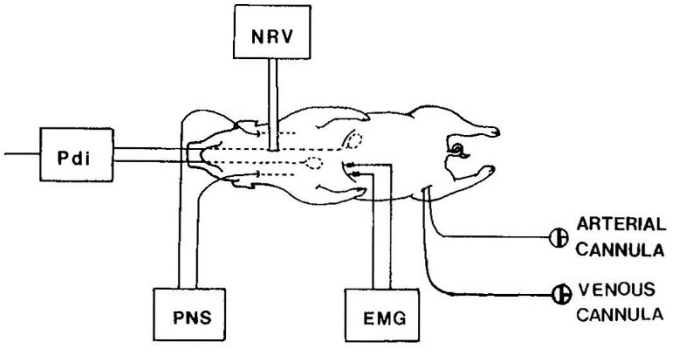

Fig. 1. Animal preparation. Piglet breathes spontaneously through a nonbreathing valve (*NRV*). Pdi is measured with two balloons as the difference between Pga and Pes pressures. Electrodes for stimulation of phrenic nerves are attached to a nerve stimulator (*PNS*). Femoral arterial and venous catheters are in place. See text for additional details.

RESULTS

The imposition of an inspiratory resistive load resulted in dramatic changes in ventilatory parameters and blood gases (Table 1). V_E decreased abruptly to approximately 50% of baseline values and did not change significantly thereafter. This change in V_E was secondary to a significant decrease in V_T at a constant respiratory rate. $PaCO_2$ reflected these changes by rising from 42.0 ± 3.7 torr at baseline to 59.8 ± 9.4 torr at 5 min and 70.2 ± 14.7 torr at 60 min of IRL. T_i was significantly prolonged during IRL compared to baseline and therefore, T_i/T_{TOT} also increased (Table 1).

Figure 2 shows the response of diaphragmatic peak integrated

Table 1. *Effect of inspiratory resistive loading on ventilatory parameters and arterial blood gases and pH (mean ± SEM for 5 piglets)*

	Baseline	5 min	60 min
V_T	35.7 ± 4.6	19.2 ± 2.7*	19.4 ± 4.7*
T_i	0.80 ± 0.07	1.47 ± 0.09*	1.30 ± 0.07*
T_i/T_{TOT}	0.23 ± 0.02	0.37 ± 0.02*	0.37 ± 0.04*
Freq	17.6 ± 2.3	15.0 ± 0.4	17.0 ± 1.2
V_E	600 ± 71	292 ± 45*	320 ± 70*
$PaCO_2$	42.4 ± 1.3	59.8 ± 4.2*	70.2 ± 7.0*
PaO_2	251 ± 34	221 ± 36	218 ± 52
pH	7.39 ± 0.02	7.27 ± 0.03*	7.22 ± 0.05*

* $p < 0.05$ compared to baseline; V_T, tidal volume in ml; T_i, inspiratory time in seconds; T_i/T_{TOT}, duty cycle; freq-frequency in breaths per minute; V_E, expired minute ventilation in ml/min; $PaCO_2$ and PaO_2 in torr units.

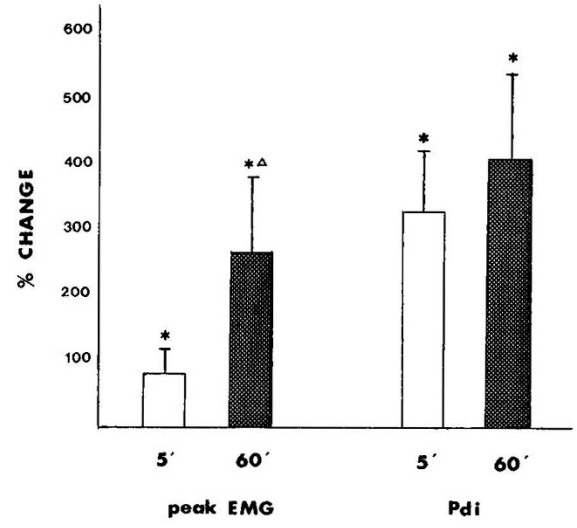

Fig. 2. Effect of inspiratory resistive loading on EMGdi and Pdi. Percent change in peak integrated diaphragmatic EMG and spontaneously generated Pdi from baseline to 5 and 60 min of inspiratory resistive loading. *Open bars* represent mean values at 5 min of IRL. *Solid bars* represent mean values at 60 min of IRL. Standard error flags are shown; n = 5. *$p < 0.05$ compared to baseline. Δ$p < 0.05$ compared to 5 min IRL.

EMG (EMGdi) and Pdi to IRL at 5 and 60 min. The peak integrated EMGdi and slope of the EMGdi from 0.1–0.4 s (slope moving average) both showed similar significant increases from 5 to 60 min of IRL indicating progressive augmentation of neural input to the diaphragm (Table 2). The ratio of force generated by the diaphragm to central neural drive to the muscle (Pdi/EMGdi) increased markedly by 5 min of IRL, but then fell at 60 min IRL. However, the ratio was still significantly higher than baseline as indicated by the results given in Figure 2.

Spontaneously generated Pdi, indicating force output of the diaphragm, increased markedly by 5 min with a smaller additional rise by 60 min which was not statistically different from the 5-min value (Fig. 2).

Force-frequency curves of the diaphragm were generated during baseline and at 60 min of IRL. Figure 3 depicts the combined force-frequency data for all five animals tested. In every animal, Pdi fell 30–57% from control values at each frequency of phrenic nerve stimulation after 60 min of IRL.

End-expiratory lung volume was measured during baseline conditions and again at 60 min of IRL in three animals. A significant decrease was noted at 60 min IRL compared to baseline (baseline: 54.7 ± 3.5 ml, 60 min IRL: 46.4 ± 2.1 ml; $p < 0.05$).

DISCUSSION

The neonatal piglet, when confronted with a large inspiratory resistive load, manifests a rapid decrease in minute ventilation that is accompanied by an increase in central nervous system output to the diaphragm. Although central drive continued to increase over the 60-min study period, the force generating ability of the diaphragm did not increase further. Moreover, when central drive to the diaphragm was controlled utilizing bilateral phrenic nerve stimulation, the ability of the muscle to generate force following 60 min of IRL was decreased compared to the baseline nonloaded period. In addition, FRC was noted to decrease during IRL breathing in association with observed expiratory abdominal contractions (Fig. 4). These abdominal contractions should increase the length and curvature of the diaphragm, tending to optimize the mechanical characteristics (24), augment transdiaphragmatic force generation (25), and minimize the decrease in force generating capacity. The decrease in the force-frequency curve of the diaphragm is therefore a conservative measure of the true decrease in force output and suggests that the neonatal piglet diaphragm fatigues during prolonged resistive loading.

Critique. This study was designed to focus on the diaphragm and therefore it was necessary to control central respiratory drive to the diaphragm and exclude the possibility of altered central nervous system output (26–28). Thus phrenic nerve stimulation was utilized. Variations of this technique have been used previously in adult humans (26) and dogs (20) to assess diaphragmatic function. This technique has been compared to direct bilateral phrenic nerve stimulation in our piglet preparation and was found to provide identical results (22).

Table 2. *Effect of inspiratory resistive loading on diaphragmatic EMG activity (mean ± SEM)*

	Baseline	5 min load	60 min load
peak EMGdi	22.8 ± 4.2	38.8 ± 6.0*	73.0 ± 14.1*,†
EMGdi SMA	23.5 ± 4.8	31.7 ± 7.8	56.3 ± 13.3*,†

* $p < 0.05$ compared to baseline.

† $p < 0.05$ compared to 5 min IRL. Peak EMGdi, peak integrated EMGdi in arbitrary units; EMGdi SMA, EMGdi slope moving average from 0.1 to 0.4 s in arbitrary units/s.

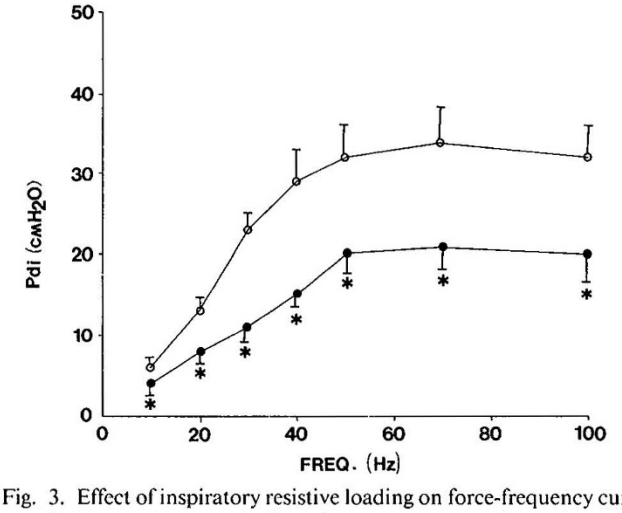

Fig. 3. Effect of inspiratory resistive loading on force-frequency curve of the diaphragm. Changes in force-frequency curves of the diaphragm after 60 min of inspiratory resistive loading. *Open circles*, mean force-frequency curve of animals under baseline conditions. *Closed circles*, mean force-frequency curve of animals after 60 min of IRL; $n = 5$. *$p < 0.01$ compared to baseline. Standard error flags are shown.

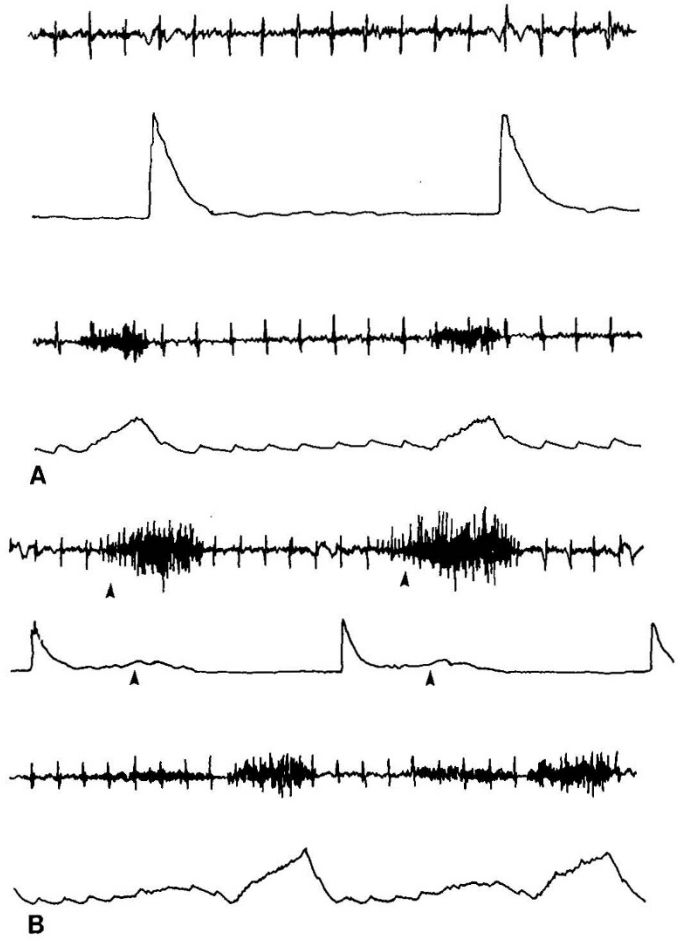

Fig. 4. Effect of inspiratory resistive loading on the abdominal muscle electrogram. A representative trace from one animal. *A*, baseline recording; *B*, tracing at 60 min of IRL. The *upper trace* is the raw EMG of the rectus abdominis muscle. The *second trace* is the expiratory flow signal. The *third trace* is the raw EMG signal from the costal diaphragm. The *fourth trace* is the integrated costal EMG signal. During inspiratory resistive loading (*B*), the abdominal muscles are recruited as manifested by activation of the rectus muscle EMG signal. Late in active expiration, the rectus muscle is activated (*arrow* on *trace 1, B*) at which time further expiratory flow is noted (*arrow* on *trace 2, B*). See text for further details.

The measurement of transdiaphragmatic pressure in this model assumes that the esophageal pressure measured as assessed by balloon manometry accurately reflects pleural pressure. We have utilized the technique of Baydur et al. (29) in this and previous studies (22, 30) to document that esophageal pressure is within 85% of mouth pressure and thus pleural pressure during occluded inspiratory efforts at end-expiratory lung volume. Occlusions during phrenic nerve stimulation gave similar results. Therefore, we feel confident that the assessment of transdiaphragmatic pressure by this technique is a reliable index of diaphragmatic force output.

Chapman *et al.* (31) found that moderately severe central nervous system hypoxia abolished the compensatory response of adult goats to resistive loading. Frantz and Milic-Emili (32) demonstrated that the response to added respiratory loads was in part secondary to hypoxemic drive. In this study, we eliminated the potentially confounding effects of mild hypoxia on the load compensation mechanisms and the deleterious effects of severe hypoxemia on the same by maintaining high arterial oxygen tensions (lowest PaO_2 − 141 torr). Therefore the likelihood that hypoxemia may have altered the compensatory response to the added resistive load has been excluded.

Ventilatory response to long-term resistive loading. The imposition of a severe inspiratory resistive load resulted in a rapid and significant decrease in minute ventilation. Within several minutes, minute ventilation dropped to approximately 50% of baseline values and did not change significantly for the remainder of the study. This change in minute ventilation was entirely due to a decrease in V_T. Although frequency of breathing remained unchanged, inspiratory time was significantly prolonged throughout the duration of loading. This is similar to findings in other studies (4, 13, 14). Abbasi *et al.* (14) recently demonstrated similar changes in preterm human infants studied during 10 min of inspiratory resistive loading (100 and 150 cm H_2O/liter/s). Studies have also been completed in adult unanesthetized sheep utilizing similar loads (<50 cm H_2O)/liter/s, 50–150 cm H_2O, and >150 cm H_2O/liter/s) (4, 13). With the low and moderate loads, the animals were able to sustain minute ventilation. However, with the severe loads (>150 cm H_2O/liter/s), the animals could compensate for variable periods of time (55–90 min), but ultimately demonstrated EMG evidence of fatigue of the diaphragm. In the present study, the resistive load utilized (650 cm/liter/s) was substantially greater than that used in other studies (4, 13, 14).

Force output and central respiratory drive. The early response (5 min) in the newborn piglet to an added inspiratory resistive load is a progressive increase in diaphragmatic force generation. Additionally, central nervous system output to the diaphragm, as assessed by integrated EMGdi activity, also progressively increased (Table 2). All EMG measures of central drive were increased over baseline both at 5 and 60 min of IRL. However, a discrepancy between force generation and the integrated EMGdi activity appeared as time passed. At 5 min of IRL, the ratio between Pdi and integrated EMGdi activity increased compared to baseline, suggesting that the central nervous system made an attempt to compensate for the added load by increasing central output which resulted in increased force generation by the diaphragm. However, by 60 min of IRL, the ratio fell. Thus, the diaphragm could no longer respond to augmented central drive and was, by definition, manifesting fatigue.

In an attempt to control central drive and therefore differentiate peripheral fatigue from central fatigue, we utilized the transvenous phrenic nerve stimulation technique. There was a significant decrease in Pdi at every frequency studied in all animals following 60 min of loaded breathing compared to baseline confirming the presence of diaphragmatic muscle fatigue. Figure 3 graphically displays the compiled data from the five animals studied. The percent decrease in transdiaphragmatic pressure varied from 30 to 57% over the range of frequencies studied. In light of the decrease in FRC at 60 min IRL, the actual drop in the force-frequency curve is likely underestimated in this study.

Hypercapnia occurred in all animals by 5 min of IRL and arterial pCO_2 did not change significantly thereafter. Previous work has examined the effects of hypercapnia on added respiratory loads. Taeusch *et al.* (33) demonstrated that the addition of carbon dioxide to the inspired gas mixture in healthy term human infants during short-term loaded breathing augmented the compensatory response. In contrast, in open-chested adult dogs Schnader *et al.* (34) found that hypercapnia ($PaCO_2$ >46 torr) significantly decreased transdiaphragmatic force generation during phrenic nerve stimulation. In this study, we cannot specifically address the superimposed effect of hypercapnia on the ventilatory response to the added resistive load. However, Watchko *et al.* (30) determined that hypercapnia of the magnitude found in the present study did not significantly effect diaphragmatic force output in this age group of animals as assessed by the force-frequency curve technique. Indeed, animals of similar postnatal age were able to compensate by decreasing end-expiratory lung volume thereby improving the length-tension relationship of the diaphragm. Our end-expiratory lung volume measurements suggest that a similar phenomenon occurred in the study piglets during IRL. Therefore, the changes in the force-frequency curves suggest that the diaphragm is fatiguing secondary to the added work load rather than depression secondary to hypercapnia.

During IRL, it was observed that the animals recruited their abdominal muscles to aid in the respiratory effort. The abdominal muscles contracted actively during the expiratory phase, especially late in expiration, and this was accompanied by expiratory gas flow from the airway. This muscle activity acts to decrease end-expiratory lung volume (Table 3) and presumably to preset the diaphragm to a more advantagous length-tension relationship. This presetting was only noted during resistive loading. This maneuver appears to allow the animal to place the diaphragm at a more advantageous length-tension relationship so the force generated during the following inspiratory effort will be greater (24, 25). To document this effort, abdominal EMG activity was examined in a qualitative fashion in two animals. Figure 4 demonstrates a representative tracing from one of the piglets. During baseline ventilation, no abdominal EMG activity was noted (Fig. 4*A*). However, as demonstrated in Figure 4*B*, the abdominal muscles are activated during expiration with a late component (*arrows*) that presets the diaphragm. These traces suggest that the abdominal contractions are increasing the length and curvature of the diaphragm therefore resulting in optimization of mechanical characteristics (2, 24, 25).

Diaphragmatic muscle functional impairment can result from multiple causes. Hypoxia (9, 11), hypercapnia (34, 35), metabolic acidosis (36), altered diaphragmatic energy metabolism (37), or substrate deficiency secondary to limitation of blood flow (5) are some of the factors thought to compromise diaphragmatic force generating capabilities. Additionally, the pattern of diaphragmatic contraction appears to play a major role in its's endurance (5, 11). This latter factor has been studied with the use of the concept of the time-tension index (5). In the present study, the possible deleterious effects of hypoxia, hypercapnia, and metabolic acidosis are not thought to account for the fatigue noted. The recent literature suggests that a major factor in the development of diaphragmatic fatigue is limitation of blood flow to the muscle and, therefore, substrate delivery (5). Since blood flow to the diaphragm increases substantially during IRL (38), the imbalance between substrate delivery and substrate needs of the diaphragm muscle require further study. We cannot address the issue of diaphragmatic blood flow in our results directly. However, the prolongation of T_i combined with a significant increase in transdiaphragmatic force generation with each breath, would lead to substantial increase in the time-tension index in these study animals and a theoretical limitation of diaphragmatic muscle blood flow. Preliminary data in the piglet model on an

identical inspiratory resistive demonstrate that blood flow to the diaphragm increases 3-fold by 30 min of IRL (39). No data exist which examine the diaphragm's substrate extraction in the newborn.

Electrophysiologic assessment of respiratory muscle function in the human neonate has suggested that the diaphragm may show evidence of fatigue. During the weaning process from ventilatory support, Lopes *et al.* (40) and Muller *et al.* (41, 42) demonstrated a shift in the high/low ratio of the diaphragm electromyographic power spectrum. This shift was taken as evidence of muscle fatigue (6, 7, 16). However, no functional assessment of respiratory muscle force generation was obtained. The possibility that central neural output alteration (central fatigue) occurred was not addressed (13, 26–28). The present study is the first assessment of functional diaphragmatic fatigue in a neonatal context.

In summary, the newborn piglet, when stressed with a long-term inspiratory resistive load, increases central nervous system drive to the diaphragm resulting in increased diaphragmatic electromyographic activity and force generation. However, as the period of loading lengthens, the diaphragm generates the same force at the cost of increasing central output. When central drive is controlled, the force-frequency curve is shifted down. These findings suggest that the diaphragm fatigues (peripheral fatigue) during long-term inspiratory resistive loading in the newborn piglet.

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