Ultrasound evaluation of piglet diaphragm function before and after fatigue

KEITH C. KOCIS, 1 PETER J. RADELL, 1 WAYNE I. STERNBERGER, 1 JANE E. BENSON, 2 RICHARD J. TRAYSTMAN, 1 AND DAVID G. NICHOLS 1

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Kocis, Keith C., Peter J. Radell, Wayne I. Sternberger, Jane E. Benson, Richard J. Traystman, and David G. Nichols. Ultrasound evaluation of piglet diaphragm function before and after fatigue. J. Appl. Physiol. 83(5): 1654–1659, 1997.—Clinically, a noninvasive measure of diaphragm function is needed. The purpose of this study is to determine whether ultrasonography can be used to 1) quantify diaphragm function and 2) identify fatigue in a piglet model. Five piglets were anesthetized with pentobarbital sodium and halothane and studied during the following conditions: 1) baseline (spontaneous breathing); 2) baseline + CO2 (inhaled CO2 to increase arterial Pco2 to 50–60 Torr (6.6–8 kPa)); 3) fatigue + CO2 (fatigue induced with 30 min of phrenic nerve pacing); and 4) recovery + CO2 (recovery after 1 h of mechanical ventilation). Ultrasound measurements of the posterior diaphragm were made (inspiratory mean velocity) in the transverse plane. Images were obtained from the midline, just inferior to the xiphoid process, and perpendicular to the abdomen. M-mode measures were made of the right posterior hemidiaphragm in the plane just lateral to the inferior vena cava. Abdominal and esophageal pressures were measured and transdiaphragmatic pressure (Pdi) was calculated during spontaneous (Sp) and paced (Pace) breaths. Arterial blood gases were also measured. Pdi(Sp) and Pdi(Pace) during baseline + CO2 were 8 ± 0.7 and 49 ± 11 cmH2O, respectively, and decreased to 6 ± 1.0 and 27 ± 7 cmH2O, respectively, during fatigue + CO2. Mean inspiratory velocity also decreased from 13 ± 2 to 8 ± 1 cm/s during these conditions. All variables returned to baseline during recovery + CO2. Ultrasonography can be used to quantify diaphragm function and identify piglet diaphragm fatigue.

inhaled carbon dioxide; diaphragm fatigue; respiratory muscle; transvenous phrenic nerve pacing; ultrasonography

DIAPHRAGM DYSFUNCTION is a prominent cause of failure to wean from mechanical ventilation (5, 12, 19, 22, 30). Several clinical tools are available to indirectly evaluate diaphragm function. Radiographic examinations (2, 4, 33–35) can be used to evaluate diaphragm location (chest radiograph, computed tomography) or motion (fluoroscopy). Phrenic nerve stimulation studies (8, 14, 20, 29, 36) have been used to assess efferent phrenic function before and after fatigue. The standards for diagnosing diaphragm weakness and fatigue (3, 12, 13, 21, 40), but the requirement for invasive catheter placement limits its utility in children. Overall, these studies have provided incomplete information about quantitative diaphragm function.

Ultrasonography has been used to image the diaphragm diagnostically in adults and children (1, 6, 7, 10, 11, 15–17, 20, 29, 36). Ultrasonography is ideal in children, because it is noninvasive, painless, safe, and portable. Image quality is superior in children compared with adults because of the small body size and lack of body fat in most children. These studies have focused on measuring superior-inferior excursion distance by two-dimensional imaging and M-mode scanning or calculating a change in diaphragm thickness. Problems exist in measuring absolute superior-inferior distances with an ultrasound probe placed on the abdomen, since the diaphragm and abdominal wall are simultaneously moving and often not in the same superior-inferior plane. Also, measuring the change in the thickness of an infant's diaphragm during inspiration is fraught with large error. We therefore sought to measure other ultrasound indexes of diaphragm function. Finally, we sought to correlate the ultrasound indexes with classic pulmonary mechanics.

A piglet animal model has been used to simulate the infant diaphragm (18, 27, 31, 32, 37). In this model, transvenous phrenic nerve pacing induces diaphragm fatigue validated by a decrease in Pdi and depletion of high-energy phosphate metabolites (8, 27, 31, 32, 37). Prior studies have suggested that a combination of low- and high-frequency fatigue is produced by this model (27). This well-established and controlled animal model of diaphragm fatigue was used in this study.

We therefore propose to use ultrasonography to quantify diaphragm function in a piglet model of diaphragm fatigue. We hypothesize that ultrasonography can be used in a piglet model to 1) quantify diaphragm function and 2) identify diaphragm fatigue.

METHODS

Animal preparation. Four- to 6-wk-old piglets (n = 5) weighing 13–19 kg were anesthetized with pentobarbital sodium (35–45 mg/kg iv or ip), and anesthesia was maintained with halothane (0.5–1.2%). All animals were cared for in accordance with National Institutes of Health guidelines (26a). After tracheostomy, animals were mechanically ventilated using an animal respirator (Harvard Apparatus, S. Natick, MA) to maintain normocarbia. Supplemental O2 was added to keep arterial PO2 >100 Torr (13.3 kPa). During the experimental protocol, CO2 was added to the inspired gas mixture to raise arterial Pco2 (Paco2) to 50–60 Torr (6.6–8 kPa) to stimulate the piglet's respiratory drive during spontaneous breathing. Catheters were placed surgically into the...
internal carotid artery for blood pressure monitoring and blood-gas sampling and into the internal jugular vein for drug and fluid administration. An air-filled (2- to 3-ml) balloon-tipped catheter was advanced through a cervical esophagotomy into the esophagus to measure esophageal pressure (Pes) as a measure of intrapleural pressure (Fig. 1). Another balloon-tipped catheter was positioned below the diaphragm into the stomach to measure abdominal pressure (Pab). Transdiaphragmatic pressure (Pdi) was calculated as Pab – Pes. These measurements were made during spontaneous breathing [Pdi(Sp)] and brief periods of phrenic nerve pacing [Pdi(Pace)] at end expiration with the airway occluded. Pdi(Pace) represents the maximum force output of the diaphragm for the preset pacing conditions and is independent of the piglet’s respiratory drive. Diaphragm fatigue was defined as a 20% decrease in Pdi(Pace) from baseline after 30 min of transvenous phrenic nerve pacing. Airway pressure (Paw) was measured via a needle-tipped catheter inserted into the endotracheal tube. Transpulmonary pressure (Ptp) was calculated as Paw – Pes. A constant Ptp at end expiration during the study periods was considered evidence of constant lung volume. Transvenous pacing catheters were placed into the external jugular veins bilaterally. Catheter position was adjusted to achieve maximum phrenic nerve pacing of the diaphragm. Brachial plexus stimulation was avoided. The pacing catheters were connected to a stimulator (model S8, Grass Medical Instruments, Quincy, MA). Pacing parameters were 2,000-ms train duration at 30 Hz, duty cycle of 0.33, and 15–25 V.

Blood-gas analysis. Arterial blood samples were obtained at regular intervals throughout the experiment. The pH, arterial PO2, P a CO2, and base deficit were analyzed with a blood-gas analyzer (ABL 3, Radiometer, Cleveland, OH).

Ultrasound evaluation. Diaphragm imaging was performed using a 128-element phased linear array transducer (3 or 5 MHz; Acuson, Mountainview, CA) in the transverse abdominal and right lateral sagittal imaging planes. The imaging planes were standardized as follows. In the transverse plane (Fig. 2), images were obtained from the midline, just inferior to the xiphoid process, and perpendicular to the abdomen. M-mode measures were made of the right posterior hemidiaphragm in the plane just lateral to the inferior vena cava immediately after discontinuation of pacing. In the right lateral sagittal plane (Fig. 3), images were obtained midway between the sagittal and coronal planes through the liver. The right posterior diaphragm was imaged at its insertion into the vertebral bodies.

From the M-mode trace (Fig. 4) obtained in the transverse plane, the mean velocity of the right posterior hemidia-
phragm during inspiration and expiration was measured. The inspiratory mean velocity ($V_i$) was calculated by dividing the distance ($D$) the right posterior hemidiaphragm moves during inspiration by the inspiratory time. Similarly, the expiratory mean velocity was calculated using $D$ and the time for expiration. A first-order time constant ($T_i$) represents the time in which 63% of inspiration (or expiration) occurs. This value ($T_i$) was then divided by the total duration of inspiration (or expiration), resulting in the inspiratory (or expiratory) time-corrected time constant ($T_{ic}$). This was done to correct for varying inspiratory (or expiratory) time in the piglets. From imaging in the right lateral sagittal plane (Fig. 3), the radius of curvature of the right posterior hemidiaphragm at end inspiration and end expiration was calculated by identifying three points on the diaphragm that defined a circle of radius $r$.

All images were recorded on videotape in super VHS format for later off-line analysis. The recordings were played through a computer with video-image capturing and digitiz-
ing capabilities (SNAPSHOT, Cardinal Technologies, Lancaster, PA). Individual images were captured, coded, and enhanced using Photo Paint (Corel, Ottawa, ON, Canada) graphics software. From the enhanced images, unique algorithms were developed within the engineering software MATLAB (Mathworks, Natick, MA) to perform the individual measurements and calculations. All measurements were recorded as the mean of three respiratory cycles and made by an investigator blinded to the individual piglet and study condition.

Study protocol. Pressure measurements during spontaneous and paced breaths, arterial blood-gas values, and ultrasound measurements were obtained during the following conditions (Fig. 5): 1) baseline-spontaneous breathing; 2) baseline + CO₂, in which piglets inhaled a CO₂ mixture to increase PaCO₂ to 50–60 Torr (6.6–8 kPa); 3) fatigue + CO₂, in which fatigue was induced with 30 min of phrenic nerve pacing while the animals inhaled the CO₂ mixture; and 4) recovery + CO₂, in which the animals recovered after 1 h of mechanical ventilation while inhaling the CO₂ mixture.

Statistical analysis. All data were analyzed using the Statistical Analysis System (SAS, Cary, NC). Analysis was performed using the paired Student's t-test, with P < 0.05 considered significant. Values are means ± SE. A priori, the primary outcome was the comparison of measurements in conditions baseline + CO₂ and fatigue + CO₂. Other comparisons were secondary outcomes performed to strengthen the validity of the study.

RESULTS

The hemodynamic, respiratory, abdominal and esophageal pressures, and ultrasound indexes for the four study conditions are shown in Table 1. The piglets' heart rate, blood pressure, and arterial blood gases did not change throughout the experiment, except for the expected increase in PaCO₂ and secondary decrease in pH when CO₂ was added to the inspired gas. Paw, Pes(Sp), and Ptp did not change for all experimental conditions, indicating constant lung volume. Pdi(Sp) decreased with fatigue + CO₂ compared with baseline + CO₂, despite nonsignificant changes in the individual components, Pab(Sp) and Pes(Sp). Pdi(Sp) normalized during recovery + CO₂. Pes(Pace), Pab(Pace), and Pdi(Pace) decreased as expected with fatigue + CO₂.

<table>
<thead>
<tr>
<th>Study Conditions</th>
<th>Baseline</th>
<th>Baseline + CO₂</th>
<th>Fatigue + CO₂</th>
<th>Recovery + CO₂</th>
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<tbody>
<tr>
<td>Heart rate, beats/min</td>
<td>124 ± 6</td>
<td>128 ± 7</td>
<td>136 ± 11</td>
<td>140 ± 10</td>
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<td>BP, mmHg</td>
<td>92 ± 6</td>
<td>92 ± 4</td>
<td>94 ± 5</td>
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<td>Systolic</td>
<td>60 ± 5</td>
<td>60 ± 5</td>
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<tr>
<td>Respiratory rate, breaths/min</td>
<td>22 ± 6</td>
<td>31 ± 11</td>
<td>25 ± 5</td>
<td>24 ± 5</td>
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<td>PaCO₂, Torr</td>
<td>41 ± 3</td>
<td>56 ± 1</td>
<td>55 ± 1</td>
<td>53 ± 2</td>
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<td>PaO₂, Torr</td>
<td>215 ± 23</td>
<td>194 ± 22</td>
<td>219 ± 16</td>
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<td>Respiratory pressures</td>
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<td>Paw, cmH₂O</td>
<td>-2 ± 0.6</td>
<td>-2 ± 0.6</td>
<td>-3 ± 0.7</td>
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<tr>
<td>Ptp, cmH₂O</td>
<td>1 ± 0.5</td>
<td>5 ± 1.3</td>
<td>1 ± 0.9</td>
<td>1 ± 0.6</td>
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<tr>
<td>Pes(Sp), cmH₂O</td>
<td>-3 ± 0.7</td>
<td>-5 ± 1.2</td>
<td>-4 ± 0.4</td>
<td>-4 ± 0.5</td>
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<td>Pab(Sp), cmH₂O</td>
<td>3 ± 1.1</td>
<td>3 ± 1.3</td>
<td>3 ± 1.1</td>
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<td>Pdi(Sp), cmH₂O</td>
<td>5 ± 0.8</td>
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<td>Pes(Pace), cmH₂O</td>
<td>24 ± 7</td>
<td>29 ± 6</td>
<td>19 ± 6*</td>
<td>13 ± 8</td>
</tr>
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<td>Pab(Pace), cmH₂O</td>
<td>20 ± 8</td>
<td>19 ± 8</td>
<td>8 ± 4*</td>
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<td>Pdi(Pace), cmH₂O</td>
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<td>49 ± 11</td>
<td>27 ± 7*</td>
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<tr>
<td>V₁, cm/s</td>
<td>12 ± 3</td>
<td>13 ± 2</td>
<td>8 ± 1.6</td>
<td>16 ± 6</td>
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<tr>
<td>Vₑ, cm/s</td>
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<td>40 ± 9</td>
<td>27 ± 6</td>
<td>55 ± 25</td>
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<td>T₁</td>
<td>0.33 ± 0.13</td>
<td>0.29 ± 0.07</td>
<td>0.29 ± 0.12</td>
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<td>0.35 ± 0.11</td>
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<td>0.34 ± 0.08</td>
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<td>0.45 ± 0.05</td>
<td>0.42 ± 0.05</td>
<td>0.45 ± 0.06</td>
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<td>Tₑ̂</td>
<td>0.57 ± 0.05</td>
<td>0.64 ± 0.07</td>
<td>0.66 ± 0.07</td>
<td>0.66 ± 0.10</td>
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<td>r₁, cm</td>
<td>36 ± 19</td>
<td>32 ± 18</td>
<td>23 ± 11</td>
<td>16 ± 5</td>
</tr>
<tr>
<td>rₑ, cm</td>
<td>13 ± 2</td>
<td>10 ± 1</td>
<td>25 ± 8</td>
<td>23 ± 7</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 5. BP, blood pressure; I, inspiratory; E, expiratory; Pace, pressure during paced breath; Pab, abdominal pressure; Paw, airway pressure; Pdi, transdiaphragmatic pressure; Pes, esophageal pressure; Ptp, transpulmonary pressure; r, radius of curvature; Sp, spontaneous breath; Tₑ, time-corrected time constant; V, mean velocity; PaO₂ and PaCO₂, arterial PO₂ and PCO₂, respectively. *P < 0.05, baseline + CO₂ vs. fatigue + CO₂, †P < 0.05 baseline vs. baseline + CO₂.

Fig. 5. Study protocol for piglet model of diaphragm fatigue. PaCO₂, arterial PCO₂.
compared with baseline + CO$_2$ and normalized during recovery + CO$_2$. In addition, there was a significant decrease in Pes(Pace) after CO$_2$ was added to the inspired gas. The only ultrasound index to significantly change during the experimental conditions was $V_I$, which decreased during fatigue + CO$_2$ compared with baseline + CO$_2$ and normalized during recovery + CO$_2$.

**DISCUSSION**

A piglet model has been developed that simulates the infant human diaphragm (8, 27, 31, 32, 37). The protocol was modified by addition of CO$_2$ to raise Pa$_{CO_2}$ to 50–60 Torr (6.6–8 kPa) in an effort to increase the piglet’s central respiratory drive. This should decrease the variability in the effort-dependent measurements [Pdi(Sp) and ultrasound indexes]. In comparing baseline with baseline + CO$_2$ conditions, pH decreased and Pa$_{CO_2}$ increased, as expected. Although a decrease in Pes(Pace) occurred, there was no decrease in Pdi(Pace) during these two conditions. There were no changes in the ultrasonographic measurements after the addition of CO$_2$.

Transvenous phrenic nerve pacing of the diaphragm has been shown to decrease force output [Pdi(Pace)] and deplete high-energy phosphate metabolites in a piglet model (8, 27, 31, 32, 37). After 30 min of pacing (fatigue + CO$_2$), there was a 34% decrease in maximal force output [Pdi(Pace)] compared with baseline + CO$_2$. Force output of the diaphragm during spontaneous breaths [Pdi(Sp)] also decreased by 25%. Only one ultrasound index, inspiratory mean velocity, was found to have decreased during these conditions. The remaining ultrasound indexes, expiratory mean velocity, inspiratory and expiratory time constants ($T_I$), inspiratory and expiratory time-corrected time constants ($T_{Ic}$), and radius of curvature during inspiration and expiration did not change.

In the recovery phase (recovery + CO$_2$), Pdi(Pace), Pdi(Sp), and $V_I$ normalized to levels found at baseline + CO$_2$. This agrees with previous work in this piglet model showing resolution of fatigue and return of high-energy phosphate metabolites after full mechanical ventilatory support and diaphragm rest (18, 27, 32).

There have been only isolated reports in animals and no studies in humans measuring the velocity of shortening of the diaphragm. The maximal velocity of shortening has been measured in isolated rat diaphragm strips under resting conditions (23). In this study the authors also found a decrease in the length of diaphragm shortening with fatigue, but the velocity was not measured under this condition (23). The association between reduced force output and reduced velocity has been described in skeletal muscle (38). Both changes may reflect altered cross-bridge function secondary to increased inorganic phosphate, increased ADP, or decreased phosphocreatine concentrations (39). We have demonstrated previously increased inorganic phosphate production and phosphocreatine consumption during fatiguing diaphragmatic contractions in this model (32).

There were several limitations of this study. First, the sample size was small ($n=5$), resulting in relatively small power, and therefore type II errors may occur. The sample size was estimated from previous results, which showed that the effects we were measuring were large. Second, our model is an acute preparation for diaphragm fatigue and not weakness. We in part attribute our inability to demonstrate a change in the diaphragm radius of curvature to this, since the diaphragm has had little time to remodel and alter its resting position. Third, central input to the diaphragm may influence the $V_I$ but was not directly measured. Instead, the possible influence of central input on $V_I$ was controlled for by addition of CO$_2$ to maximize respiratory drive and by addition of a recovery phase to the experimental protocol. Finally, we have not validated the $V_I$ to an absolute rate of diaphragm shortening in the anterior–posterior direction. Additional experiments are required to measure simultaneously the velocity of the diaphragm in the anterior–posterior direction and the $V_I$. Until these data are acquired, we are unable to state that $V_I$ was an actual velocity or whether it only positively correlated with the velocity of shortening of the diaphragm in the anterior–posterior direction.

In summary, quantitative ultrasound measurements, specifically $V_I$, can be used to evaluate diaphragm function in a piglet model of diaphragm fatigue. We speculate that these techniques could be applied to the evaluation of the diaphragm in children. If these techniques are successful, we expect that this tool will enable investigators to better evaluate the function of the diaphragm. By quantifying diaphragm function, one might be able to predict the timing for successful extubation from mechanical ventilation, evaluate diaphragm function during drug therapy, and assess mechanical ventilation strategies designed to strengthen the diaphragm.

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