Effects of loaded breathing and hypoxia on diaphragm metabolism as measured by 31P-NMR spectroscopy

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Radell, Peter J., Scott M. Eleff, and David G. Nichols. Effects of loaded breathing and hypoxia on diaphragm metabolism as measured by 31P-NMR spectroscopy. J. Appl. Physiol. 88: 933–938, 2000—Diaphragm fatigue may contribute to respiratory failure. 31P-nuclear magnetic resonance spectroscopy is a useful tool to assess energetic changes within the diaphragm during fatigue, as indicated by Pi accumulation and phosphocreatine (PCr) depletion. We hypothesized that loaded breathing during hypoxia would lead to diaphragm fatigue and inadequate aerobic metabolism. Seven piglets were anesthetized by using halothane inhalation. Diaphragmatic contractility was assessed by transdiaphragmatic pressure (Pdi) at end expiration with the airway occluded. A nuclear magnetic resonance surface coil placed under the right hemidiaphragm measured Pi and PCr during four conditions: control, inspiratory resistive breathing (IRB), IRB with hypoxia, and recovery (IRB without hypoxia). IRB alone resulted in hypercarbia (32 ± 7 to 61 ± 21 Torr) and respiratory acidosis but no change in diaphragm force output or aerobic metabolism. Combined IRB and hypoxia resulted in decreased force output (Pdi decreased by 40%; from 30 ± 17 to 19 ± 11 mmHg) and evidence of metabolic stress (ratio of Pi to PCr increased by 290%; from 0.19 ± 0.09 to 0.74 ± 0.27). We conclude that diaphragm fatigue associated with inadequate aerobic oxidative metabolism occurs in the setting of loaded breathing and hypoxia. Conversely, aerobic metabolism and force output of the diaphragm remain unchanged from control during loaded normoxic or hyperoxic breathing despite the onset of respiratory failure.

inspiratory resistive breathing; respiratory muscle fatigue; transdiaphragmatic pressure; high-energy phosphates; energetics; phosphorus-31 nuclear magnetic resonance

RESPIRATORY FAILURE IS A COMMON finding in critically ill patients. Respiratory pump failure, defined as the inability to prevent CO₂ retention, is often involved. Although mechanisms of pump failure are not fully understood, respiratory muscle dysfunction may be an important component in this process (23). The diaphragm is the major muscle of the respiratory system, and diaphragm fatigue may be of importance in the development of pump failure, contributing to acute respiratory failure and the inability to wean from mechanical ventilation (5, 20). Mechanisms proposed to explain the development of diaphragm fatigue include inadequate central activation, decreased neuromuscular conduction, peripheral muscle fatigue, and combinations of these. Study of the mechanisms of respiratory muscle fatigue and the interactions of peripheral muscle and the central control of ventilation have received high priority in the National Heart, Lung, and Blood Institute Workshop Summary on respiratory muscle fatigue (19).

The in vivo study of oxidative metabolism has been enhanced in recent years by the use of 31P-nuclear magnetic resonance (NMR) spectroscopy. This technique allows semicontinuous measurement of high-energy phosphates (3) and has been employed in studies of skeletal and cardiac muscle metabolism, but little has been published on in vivo diaphragm muscle metabolism.

After phrenic nerve stimulation in a piglet model, decreased force output correlates well with depleted high-energy phosphates in the diaphragm, indicating peripheral muscle fatigue (15). In contrast, evidence in a spontaneously breathing model with inspiratory resistive loading showed that respiratory failure with hypercapnea and respiratory acidosis occurs before the development of decreased force output or substrate depletion in the diaphragm (18). This finding agrees with other recent work (21) and supports the role of central and/or reflex mechanisms that affect the breathing pattern in response to resistive loading. Thus the role of peripheral fatigue remains unclear and may vary depending on the pathophysiological perturbations occurring in the clinical setting.

The effect of hypoxia on development of respiratory muscle fatigue is unclear, varying depending on the study (2, 4, 10, 12). To our knowledge no study has studied measures of diaphragm energetics repetitively in an attempt to correlate changes in diaphragm contractility during hypoxic inspiratory resistive breathing (IRB) with changes in high-energy-phosphate metabolism.

The present study was designed to study diaphragm metabolism via NMR spectroscopy in the presence of inspiratory resistive breathing and hypoxia. We have asked whether peripheral diaphragm fatigue associated with inadequate oxidative metabolism occurs in a model of IRB and superimposed hypoxia. This information might in turn shed light on the relationship between central and peripheral pump failure in the development of respiratory failure.

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METHODS

Animal preparation (Fig. 1). Seven piglets, age 4–6 wk, weighing 13–17 kg, were initially anesthetized with pentobarbital sodium (35–45 mg/kg ip). After cervical dissection and tracheostomy, anesthesia was maintained with halothane inhalation (0.5–1.0%). The animals were mechanically ventilated for the remainder of the preparation phase via an animal respirator (Harvard Apparatus, South Natick, MA) with supplemental oxygen to maintain an arterial \( \text{PCO}_2 \) of 35–45 Torr (4.7–6.0 kPa) and an arterial \( \text{PO}_2 \) (\( \text{Pao}_2 \)) >100 Torr (13.3 kPa). A catheter was placed in the left internal carotid artery for blood pressure monitoring and blood-gas determinations. A second catheter was placed in the internal jugular vein for maintenance fluid and drug administration. An air-filled (2-ml), balloon-tipped catheter was advanced through a cervical esophagotomy to the midesophagus to measure esophageal pressure (Pes). The catheter length was estimated from external measurements, and placement was checked for cardiac oscillations and then withdrawn slightly to ensure placement in the midesophagus. Placement was verified at autopsy. Airway pressure (Paw) was measured via a needle-tipped catheter inserted into the endotracheal tube. A midline laparotomy was performed, and an identical balloon-tipped catheter was positioned under the diaphragm in the left upper quadrant and used to measure abdominal pressure (Pab). The abdomen was then closed in layers.

Pressure measurements were recorded via air-filled pressure transducers on a Gould strip-chart recorder (Gould Electronics, Cleveland, OH).

An elliptical (4 \( \times \) 2 cm) NMR coil was positioned in contact with the abdominal surface of the costal right hemidiaphragm. The coil was wrapped in plastic film to protect it from moisture and was shielded from the intercostal muscles and the liver by foam padding and gauze. The coil was secured in place by suturing the coil cable to the abdominal wall. Initial scans at times revealed improper placement requiring repositioning of the coil before the protocol was started, but little movement artifact was seen during the course of the experiments.

A 7-Fr bipolar transvenous pacing catheter (Mansfield Instruments, Mansfield, MA) was placed in each external jugular vein. Catheter position was adjusted to achieve phrenic nerve pacing of the diaphragm. Brachial plexus stimulation was avoided and diaphragm contraction verified by observation and palpation of the diaphragm through the laparotomy during stimulation. Transvenous pacing allowed us to achieve diaphragm pacing without a thoracotomy, thus making periods of spontaneous respiration feasible. The pacing catheters were connected to a Grass S8 stimulator (Grass Medical Instruments, Quincy, MA). Each animal was placed in a similar supine position in the magnet for all measurements, minimizing the effect of varying position on muscle recruitment or blood flow between experiments. An oxygen analyzer (model OM 15, Sensormedics, Anaheim, CA) was placed in the inspiratory limb of the breathing circuit for continuous measurement of the inspired oxygen fraction (\( \text{FIO}_2 \)). Hypoxia was achieved by adding nitrogen to the inspiratory limb and titrating to the desired \( \text{FIO}_2 \). Arterial blood samples were obtained at regular intervals throughout the experiment, including at the beginning and end of each study period. The pH, \( \text{PaO}_2 \), \( \text{PCO}_2 \), and base deficit were analyzed with a Radiometer ABL 3 (Radiometer America, Cleveland, OH). Time-control experiments showing the stability of this preparation for up to 4 h have been described previously (15).

Study protocol (Fig. 2). The study protocol consisted of four study periods: control, IRB, IRB with hypoxia, and recovery. After the surgical preparation, the animals were positioned supine in the magnet during continued mechanical ventilation and anesthesia. After the 20-min control period the animals were switched to a spontaneous breathing circuit. Each animal then underwent a period of IRB avoiding hypoxia, followed by a period of IRB and hypoxia, and finally a recovery period with IRB and normoxia-hyperoxia. The inspiratory resistive load consisted of a 2.0-mm-inner-diameter, 12-cm-long endotracheal tube in the inspiratory limb of the circuit. The inspiratory and expiratory limbs were separated using a Hans-Rudolph valve (model 1700, Kansas City, MO). Resistance equaled 190 cmH\(_2\)O·l\(^{-1}\)·min at a flow of 2 l/min. This was found in preliminary experiments to be the maximum resistance tolerated by the animals without provoking respiratory arrest. In all but one animal this level of resistance led to a Pdi during spontaneous IRB breathing that was >60% of the maximum Pdi achieved by pacing.

The initial IRB period was 30 min. Hypoxia was then achieved by titrating increasing flow rates of nitrogen into the inspiratory limb until the \( \text{FiO}_2 \) was in the 0.09–0.14 range, corresponding to arterial saturation between 39 and 50%, and \( \text{Pao}_2 \) was between 26 and 40 Torr (3.5 and 5.3 kPa). The hypoxia period varied depending on the time required to achieve the desired \( \text{FiO}_2 \) lasting between 45 and 90 min. The recovery period was 30 min. The study was approved by our Institutional Review Board for the care of animal subjects, and handling and care of the animals were in accordance with...
Transdiaphragmatic pressure (Pdi) was calculated as the difference between Pab and Pes. Pdi was measured via supramaximal phrenic nerve stimulation of the diaphragm at end expiration. National Institutes of Health guidelines for ethical animal experimentation were followed. The experiments were approved by the institutional animal care and use committee. The diaphragm was paced for two to three contractions and spontaneous Pes, Pab, and Paw were monitored continuously. Train duration 2,000 ms, respiratory rate 10 breaths/min, and inspiratory :expiratory ratio 1:1 were used previously (16) and shown to not compromise diaphragm fatigability. This ensured near constant shape and geometry of the diaphragm before stimulation as well as constant activation of the diaphragm independent of central activation. Diaphragm fatigue was defined as a fall in Pdi of >2% from baseline during pacing.

The spontaneous respiratory rate decreased significantly with the application of the inspiratory resistance, decreased further during the period of hypoxia, and 10.2±0.3 Torr in the IRB group. MAP was unchanged during the studies. There was no significant change in inspired halothane concentration or Ptp during the experiments. The spontaneous respiratory rate decreased significantly with the application of the inspiratory resistance, decreased further during the period of hypoxia.

Table 1. Effects of loaded breathing and hypoxia on respiratory parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>IRB</th>
<th>IRB + Hypoxia</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>PpBar, mmHg*</td>
<td>5.7±3.1</td>
<td>4.8±2.0</td>
<td>3.7±1.3</td>
<td>4.5±1.5</td>
</tr>
<tr>
<td>PpEs, mmHg*</td>
<td>-24±13</td>
<td>-23±8</td>
<td>-16±10</td>
<td>-23±8</td>
</tr>
<tr>
<td>Pdi, mmHg*</td>
<td>30±17</td>
<td>27±8</td>
<td>19±11</td>
<td>27±10</td>
</tr>
<tr>
<td>Ptpi, mmHg*</td>
<td>-10±5</td>
<td>5±16</td>
<td>-10±8</td>
<td>1.5±6</td>
</tr>
<tr>
<td>P/Pcr</td>
<td>0.19±0.1</td>
<td>0.29±0.04</td>
<td>0.74±0.27†</td>
<td>0.34±0.11</td>
</tr>
<tr>
<td>pH</td>
<td>7.46±0.07</td>
<td>7.25±0.08†</td>
<td>7.26±0.05†</td>
<td>7.22±0.06†</td>
</tr>
<tr>
<td>PaCO2, Torr</td>
<td>32±7</td>
<td>61±21†</td>
<td>60±10†</td>
<td>60±13†</td>
</tr>
<tr>
<td>PaO2, Torr</td>
<td>222±43</td>
<td>236±99</td>
<td>212±87</td>
<td>212±87</td>
</tr>
<tr>
<td>SaO2, %</td>
<td>99±3</td>
<td>98±3</td>
<td>98±3†</td>
<td>98±3</td>
</tr>
<tr>
<td>FIO2</td>
<td>0.60±0.17</td>
<td>0.77±0.15</td>
<td>0.12±0.03*</td>
<td>0.77±0.13</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>99±13</td>
<td>102±11</td>
<td>98±13</td>
<td>97±8</td>
</tr>
<tr>
<td>%Halothane</td>
<td>9.0±0.1</td>
<td>9.0±0.2</td>
<td>0.7±0.4</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Respiratory rate, breaths/min</td>
<td>31±10</td>
<td>24±10†</td>
<td>17±9†</td>
<td>24±9</td>
</tr>
</tbody>
</table>

Values are means ± SD. IRB, inspiratory resistive breathing; p, paced breathing; s, spontaneous breathing; Pab, abdominal pressure; Pes, esophageal pressure; Pdi, transdiaphragmatic pressure; Ptp, transpulmonary pressure; Pcr, phosphocreatine; PaCO2, arterial PCO2; PaO2, arterial PO2; SaO2, arterial O2 saturation; FIO2, inspired oxygen fraction; MAP, mean arterial pressure. *Pressure resulting from a paced contraction of the diaphragm at the end of the period. †P < 0.05 compared with control.

RESULTS

The results are shown in Table 1. Heart rate and MAP were unchanged during the studies. There was no significant change in inspired halothane concentration or Ptp. The spontaneous respiratory rate decreased significantly with the application of the inspiratory resistance, decreased further during the period of hypoxia.
and then returned to the IRB level during recovery. IRB resulted in a significant rise in PaCO₂ and lowering in arterial pH, which then remained stable during the periods of IRB with hypoxia and recovery.

The paced Pdi as measured by phrenic nerve pacing decreased by 40% during the period of IRB and hypoxia (27 ± 8 to 19 ± 11 mmHg), consistent with our definition of fatigue (Fig. 3). This fall was due in large part to the fall in paced Pes, which decreased to 66% of control (−23 ± 8 to −16 ± 10 mmHg). The paced Pab also fell to 65% of control, but this was a much smaller change in absolute terms. In one animal the paced Pdi increased from control to IRB. One explanation for this would be that lung volume was not at FRC during the initial measurement, possibly resulting in altered diaphragm shape and contractile potential.

The spontaneous Pdi increased in response to the inspiratory load and remained relatively constant during hypoxia and recovery. Interestingly, the paced Pdi did not exceed the spontaneous Pdi during IRB with hypoxia, suggesting that, for the given level of activation, force output was limited by factors intrinsic to the diaphragm.

Oxidative metabolism of the diaphragm, as measured by the P_i/PCr, did not change significantly during the period of IRB. In contrast, IRB and hypoxia did result in a significant change, because the P_i/PCr increased from 0.19 ± 0.09 to 0.74 ± 0.27, an increase of 290% (Fig. 4). During recovery the P_i/PCr returned to the IRB level. In one animal magnetic resonance data were not obtained during recovery because there was relatively little metabolic change from IRB to IRB with hypoxia, such that little change during recovery could be expected. Serial 31P-NMR spectra from one experiment are shown in Fig. 5.

**DISCUSSION**

Main findings. The main findings of this study are 1) respiratory failure, defined as hypercarbia and respiratory acidosis, occurs before evidence of peripheral diaphragm fatigue during loaded breathing; 2) the addition of hypoxia to loaded breathing results in decreased force output capacity of the diaphragm; and 3) the reduction in force output capacity during hypoxic loaded breathing is associated with inadequate oxidative metabolism of the diaphragm. We conclude that the combination of loaded breathing and severe hypoxia results in peripheral diaphragm fatigue, which may in part be due to inadequate oxidative metabolism of the diaphragm.

Role of hypoxia and IRB. The relationship of hypoxia and inspiratory loading to the onset of respiratory failure has been examined by other authors. As early as 1981, Aubier et al. (1) found that hypoxia hastened the onset of respiratory muscle fatigue, but that study did not examine whether this resulted from central or peripheral mechanisms. Bark et al. (2), using an in vitro preparation, found diaphragm fatigue during the combination of severe hypoxia and fatiguing tension-time index, whereas fatigue did not occur during iso-
lated hypoxia or increased load, and the study obviously did not examine spontaneous respiration. In contrast, a study in 1-mo-old piglets exposed to IRB and moderate hypoxia found no contribution of hypoxia to fatigue and no effect of hypoxia on metabolic state as assessed by single biopsy (14). More recently, Ciuf et al. (4) used a decerebrate rat model that eliminated central input and found evidence for peripheral muscle fatigue in response to inspiratory loading and postulated a possible role for oxygen-derived free radicals in the development of fatigue. Our study supports the contribution of IRB and hypoxia to respiratory failure and decreased diaphragm contractility during spontaneous breathing.

Metabolism and peripheral fatigue. The role of inadequate oxidative metabolism as a cause of skeletal muscle fatigue is clear. An analogous role for inadequate metabolism causing peripheral diaphragm fatigue, which contributes to respiratory failure, has not been proven as clearly. Nichols et al. (15) found that diaphragm fatigue caused by phrenic nerve pacing was associated with inadequate oxidative metabolism, suggesting an imbalance of diaphragm energy supply and demand. A further study comparing paced diaphragm contractions with loaded spontaneous breathing confirmed the association of peripheral fatigue with inadequate metabolism during pacing but did not find evidence for peripheral diaphragm fatigue during normoxic spontaneous breathing (18). Ferguson et al. (6) also found a relationship during pacing between contractile fatigue of the diaphragm and biochemical changes, indicating imbalance in muscle energetics (diaphragm glycogen depletion and lactate accumulation) but did not find these changes during spontaneous loaded breathing. The present study shows a relationship between peripheral contractile diaphragm fatigue and inadequate oxidative metabolism and seems to provide evidence for the first time for the contribution of peripheral muscle fatigue in failure of the respiratory system to maintain adequate gas exchange in the setting of spontaneous breathing with IRB and hypoxia. The fact that diaphragm fatigue can be attenuated in both an animal model (11) and clinical setting (22) by peripherally acting drugs also supports some component of peripheral fatigue.

Peripheral vs. central fatigue. The relative importance of this peripheral fatigue remains unclear. Although no measurement of central activation of the diaphragm has been made in this study, the results tend to support the role of central input in the development of respiratory failure before peripheral fatigue, in agreement with studies by Kanter and Fordyce (13) and Watchko et al. (24). These results are further supported by a recent study by Sassoon et al. (21) in which rabbits exposed to IRB developed respiratory failure with hypercarbia and hypoxia before diaphragm fatigue. Analogously, in the present study peripheral diaphragm fatigue becomes manifest after respiratory failure has already occurred. Interestingly, at this point the spontaneous Pdi is roughly the same as the paced Pdi. This seems to indicate that force output during IRB and hypoxia is near maximum for the given level of activation, and that force output is limited by inadequate peripheral oxidative metabolism. We speculate that central activation decreases initially, even to the point of respiratory failure, in effect decreasing metabolic demands and preventing peripheral fatigue, until the additional stress of hypoxia overwhelms this defense mechanism and leads to peripheral fatigue, evidenced by decreased diaphragm contractility.

If our results can be extrapolated to the clinical setting, it appears that hypercapneic respiratory failure may arise in response to an inspiratory load in the absence of diaphragm fatigue. Diaphragm fatigue in association with inadequate oxidative metabolism contributes to pump failure with the addition of a second stress in the form of severe hypoxia. We speculate that feedback mechanisms between the diaphragm and the brain stem respiratory center regulate diaphragm force output to preserve aerobic diaphragm metabolism rather than blood-gas homeostasis in the setting of a normoxic-hyperoxic inspiratory load. These protective mechanisms may be overwhelmed in the face of severe hypoxia.

Limitations. Several methodological limitations of this study should be observed. Although the present studies could not have been accomplished without anesthesia, there are obvious differences in the response of halothane-anesthetized animals to those which might be seen in an awake spontaneously breathing model (17). Because the structure and function of the diaphragm vary considerably with age and among species, broader generalization of these results from immature piglet diaphragm must be made with caution.

The protocol required a small laparotomy for placement of the NMR coil. Despite careful closure in layers, it is possible that diaphragm mechanics as well as activation are affected by the surgical preparation.

During periods of control breathing and IRB, we did not distinguish between normoxia and hyperoxia. Recent work has shown, however, that variation in F1O2 outside the hypoxic range may influence cell metabolism (9). Whether this difference could affect the response to hypoxia is unknown.

It could be argued that the observed diaphragm fatigue resulted from the prolonged IRB rather than the combination of IRB with hypoxia. Randomization was not used as we have previously studied IRB alone and were only interested in this study in imposition of hypoxia on existing IRB. In previous control studies, periods of up to 4 h of IRB were tolerated without signs of fatigue. Finally, the fact that the P/PiCr improved during recovery, whereas IRB was maintained, also indicates the importance of IRB together with hypoxia as the cause of diaphragm fatigue. Because hypoxia was only studied superimposed on IRB, we cannot be certain that our findings did not result solely from the hypoxia regardless of the inspiratory load, but previous work indicates that this is not the case (2).

The P/PiCr reached one or greater in several but not all animals. Although substrate depletion occurred in
several animals, explaining the decrease in force output, in other animals the P/PCr indicates metabolic stress without evidence of substrate depletion. Although the regulatory role of decreased PCr and increased P, in leading to decreased force output remains unclear (7), it may be that metabolic stress is sufficient to contribute to decreased force output of the diaphragm.

Factors other than inspiratory loading and hypoxia may have contributed to the observed diaphragm fatigue. The hypercarbia observed approaches levels that may have negative affects on diaphragm function (25). However, the fact that Pdi returned to control levels during recovery, despite persistent hypercapnia, argues against a significant contribution of hypercarbia to fatigue.

Conclusions. In summary, inspiratory resistive breathing in the absence of hypoxia results in respiratory failure without impairment of diaphragm force output or metabolism, suggesting decreased central activation. Conversely, the addition of severe hypoxia to inspiratory resistive breathing results in peripheral diaphragm fatigue and inadequate oxidative metabolism of the diaphragm in this in vivo piglet model.

The authors gratefully acknowledge the assistance of Dr. Vadappuram Chaddock in acquiring nuclear magnetic resonance spectra for several of the experiments. This work was presented in part at American Thoracic Society International Conference, May 17–21, 1997. Address for reprint requests and other correspondence: D. G. Nichols, Dept. of Anesthesiology and Critical Care Medicine, The Johns Hopkins Hospital, 600 N. Wolfe St., Baltimore, MD 21287-3711. Received 16 December 1998; accepted in final form 8 November 1999.

REFERENCES