

# Diaphragmatic Response Is Influenced by Previous Muscle Activity

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**OBJECTIVE:** Previous muscle activity can alter muscle contractility and lead to strength underestimation or overestimation in functional measurements. The objective of this study was to evaluate changes in the maximum pressure produced by the diaphragm after different series of spontaneous near-to-maximal isometric contractions.

**METHODS:** Duplicate studies were performed on 6 dogs with a mean (SD) weight of 26 (7) kg. The supramaximal response of the diaphragm was achieved by simultaneous supramaximal stimulation of both phrenic nerves, both under basal conditions and after series of 5, 10, and 20 spontaneous inspiratory efforts against the occluded airway, performed before and after spinal anesthesia (which eliminates the ventilatory contribution of the intercostal muscles). The response was measured using the twitch gastric pressure (Pga) and twitch esophageal pressure (Pes) and by muscle shortening (sonomicrometry).

**RESULTS:** The short series of 5 inspiratory efforts and, in particular, the medium series of 10 efforts produced potentiation of the contractile response, with a rise in the Pga from 3.2 (0.4) cm H<sub>2</sub>O to 3.7 (0.3) cm H<sub>2</sub>O, and from 3.5 (0.3) cm H<sub>2</sub>O to 3.9 (0.3) cm H<sub>2</sub>O, respectively ( $P=0.05$  in both cases). The potentiation was somewhat greater after subarachnoid anesthesia (an increase in the Pga of 21% after the medium series of 10 efforts with anesthesia vs 11% without anesthesia). However, the long series of 20 efforts produced a fall in the response, with a decrease in the Pga from 3.2 (0.4) cm H<sub>2</sub>O to 2.5 (0.3) cm H<sub>2</sub>O ( $P<0.05$ ), probably due to fatigue overcoming the effect of potentiation.

**CONCLUSIONS:** Previous effort affects the contractile capacity of the diaphragm and it is difficult to predict the predominance of fatigue or potentiation in the response. This factor must be taken into account when determining

the maximum respiratory pressures in daily clinical practice.

**Key words:** *Respiratory muscles. Potentiation. Fatigue. Respiratory pressures.*

## Respuesta diafragmática en función de la actividad previa del músculo

**OBJETIVO:** La actividad previa puede modificar la contractilidad muscular, lo que puede conducir a la infra o supraestimación de la fuerza en las determinaciones funcionales. El presente trabajo se ha propuesto como objetivo valorar cambios en la presión máxima generada por el diafragma tras diferentes series de contracciones isométricas espontáneas y cuasi máximas.

**MÉTODOS:** Se estudiaron por duplicado 6 perros con un peso medio  $\pm$  desviación estándar de 26  $\pm$  7 kg. Se obtuvo la respuesta supramáxima del diafragma —presiones gástrica (Pga<sub>tw</sub>) y esofágica (Pes<sub>tw</sub>) inducidas por estimulación frénica bilateral, y acortamiento muscular (sonomicrometría)— por estimulación simultánea supramáxima de ambos nervios frénicos, tanto en situación basal como tras series cortas (5), medianas (10) y largas (20) de esfuerzos inspiratorios espontáneos contra la vía aérea ocluida, antes y después de administrar anestesia subaracnoidea (elimina la contribución ventilatoria de los músculos intercostales).

**RESULTADOS:** La serie corta y, sobre todo, la serie mediana provocaron la potenciación de la respuesta contráctil (Pga<sub>tw</sub> de 3,2  $\pm$  0,4 a 3,7  $\pm$  0,3, y de 3,5  $\pm$  0,3 a 3,9  $\pm$  0,3 cmH<sub>2</sub>O, respectivamente;  $p < 0,05$  ambas). La potenciación fue algo superior con anestesia subaracnoidea (un 21 frente al 11% sin anestesia, para la Pga<sub>tw</sub> tras las series medianas). La serie larga provocó, sin embargo, una disminución de la respuesta (Pga<sub>tw</sub>: 3,2  $\pm$  0,4 a 2,5  $\pm$  0,3 cmH<sub>2</sub>O;  $p < 0,05$ ), probablemente por predominio de la fatiga sobre la potenciación.

**CONCLUSIONES:** Los esfuerzos previos determinan la capacidad contráctil del diafragma y resulta difícil predecir el predominio de fatiga o de potenciación en la respuesta. Este factor debería tenerse en cuenta al determinar las presiones respiratorias máximas en la clínica diaria.

**Palabras clave:** *Músculos respiratorios. Potenciación. Fatiga. Presiones respiratorias.*

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TABLE 1  
Basic Characteristics of the Phenomena of Muscle Potentiation, Facilitation, Fatigue, and Weakness

	Definition	Response	Duration	Recovery	Biologic Basis
Postactivity potentiation	Transitory increase in maximum muscle response due to previous activity	+++	10 s-20 min	Rest	Slow return of calcium to the cisterns of the sarcoplasmic reticulum
Facilitation	Momentary increase in the maximum response of the muscle if the stimulus is applied after voluntary contraction has started	+	Momentary	Rest	Release of additional Ca <sup>2+</sup> and neurotransmitters
Fatigue	Temporary reduction or absence of response due to intense previous muscle activity	-/-	30 min-1 h	Rest	Depletion of Ca <sup>2+</sup> and energy elements
Weakness	Stable reduction of the response due to intrinsic muscle disorders and/or deconditioning	-	Long periods	Treat the cause. Training	Changes in the fibers, enzyme disturbances, local oxidative stress, local inflammation, etc

**Introduction**

At rest, the main respiratory muscle in healthy individuals is the diaphragm. It is formed of 2 embryologically, anatomically, and functionally different parts—crural and costal. The crural part basically fixes the muscle to the

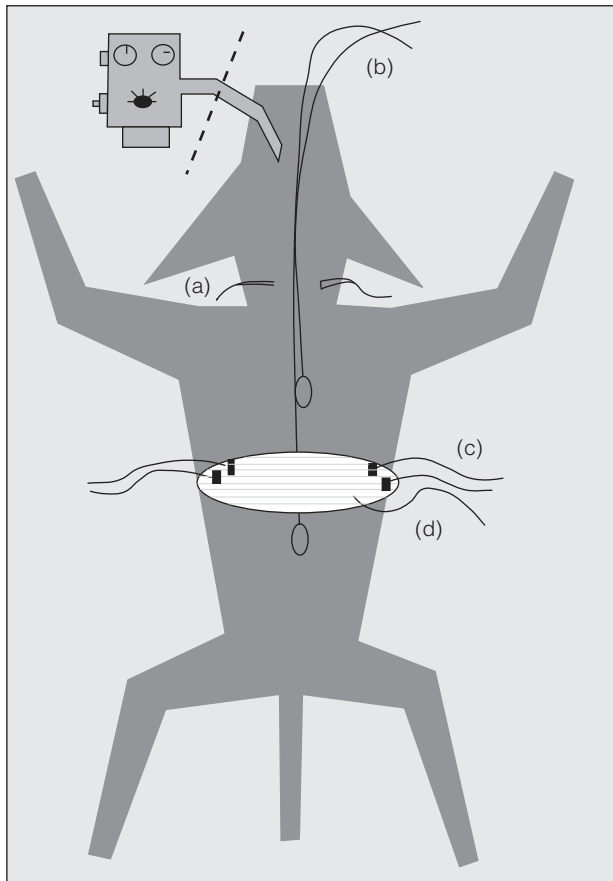
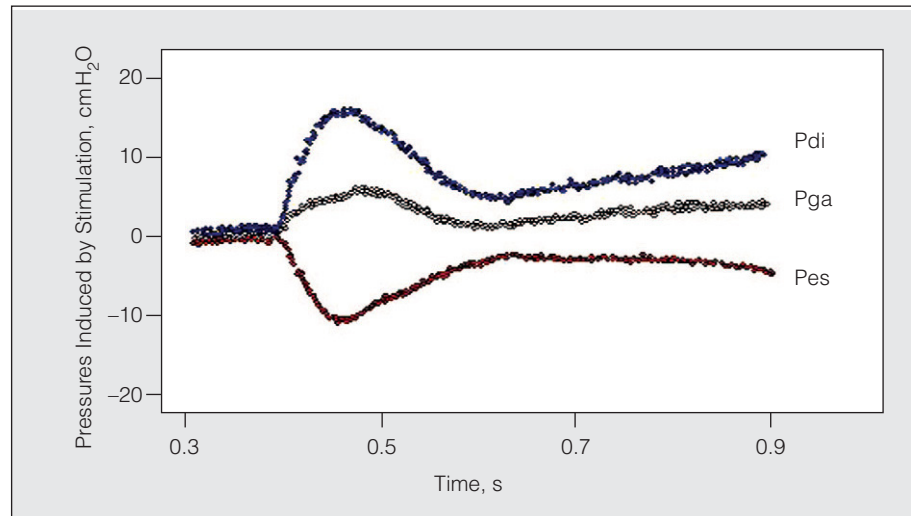


Figure 1. Diagram of the model used in the present study, showing the stimulation electrodes (a), esophageal and gastric balloon catheters (b), and the sonomicrometry piezoelectric crystals (c) and electromyography electrodes (d) implanted on the costal diaphragm.

adjacent structures, whereas the costal part acts as a piston that, on contracting, increases the size of the thoracic cavity. However, other muscles, such as the parasternal muscles (part of the internal intercostal muscles) and the external intercostal muscles (particularly the more cephalad and anterior ones) also participate in inspiration.<sup>1</sup> Evaluation of the functional properties of the respiratory muscles is useful both in intrinsically respiratory diseases and in other conditions. However, as the contractile force cannot be measured directly in clinical practice, it is evaluated using an approximation from the pressures generated by the respiratory muscles.<sup>1,2</sup> These measurements can be performed by use of voluntary maneuvers or by stimulation of the muscle or corresponding nerves.<sup>3-6</sup> In either case, measurements are usually repeated a number of times in order to confirm their reproducibility and the validity of the maximal measurement.<sup>7</sup> If the aim is also to eliminate a learning effect, several series of maneuvers should be performed before the one considered valid.<sup>7,8</sup> However, this practice can introduce the possibility that previous activity leads to changes in the maximum contractile response. It is known that previous contractions can increase muscle response (a phenomenon known as postactivation potentiation),<sup>9</sup> but if previous activity was intense, muscle response can decrease due to fatigue.<sup>1,10</sup> In the case of less intense but persistent overactivity, some authors consider that chronic muscle fatigue can develop,<sup>11</sup> leading to a situation in which the muscle can perform its function but with certain deficiencies. If, in contrast, there is a marked and prolonged decrease in activity, deconditioning can result. The solution to fatigue is rest; deconditioning, on the other hand, will lead to weakness, and the muscle will require training. Table 1 shows the main differences between these phenomena.

Our hypothesis was that previous activity would modify diaphragmatic response to stimulation: mild activity would potentiate the response, while more prolonged, intense activity would reduce it, due to the onset of fatigue. The objective of this experimental study in an animal model<sup>12-14</sup> was to evaluate the maximum contractile response of the diaphragm after different series of submaximal efforts.



**Figure 2.** Graph of the principal respiratory pressures (gastric and esophageal pressures and transdiaphragmatic pressure calculated from the 2 previous signals) obtained during supramaximal twitch stimulation of the diaphragm. Pdi indicates transdiaphragmatic pressure; Pes, esophageal pressure; Pga, gastric pressure.

## Methods

### Animal Model (Figure 1)

Six mongrel dogs with a mean (SD) weight of 26 (7) kg were anesthetized with 25 mg/kg pentobarbital sodium intravenously, followed by inhaled anesthesia with halothane. After insertion of esophageal and gastric balloon catheters, upper abdominal laparotomy was performed to insert piezoelectric crystals (sonomicrometry) and surface electrodes on the costal diaphragm. The dogs were kept in the supine position at all times, warmed by surgical lamps (rectal temperature of 37°C), and were ventilated mechanically (20–25 ml/kg, 14 cycles/min; Mark-8 Respirator, Bird Corporation, Palm Springs, California, USA). After closure of the laparotomy in 2 planes, the inhaled anesthesia was interrupted and the dogs started to breathe spontaneously through the endotracheal tube. During this phase, the animals were maintained with low doses of pentobarbital, at a level that blocked the corneal reflex. Spinal (subarachnoid) anesthesia was then administered in order to eliminate the contribution of the intercostal muscles to the inspiratory effort. The procedures used in the protocol were approved by the animal research ethics committee of Hôpital de Notre Dame, and efforts were made at all times to use the minimum number of animals required and to avoid any possible discomfort. The sample size was calculated from previous studies.<sup>12,14</sup>

**Electrical stimulation.** Two silver electrodes were placed around the phrenic nerves (at the level of T2–T3), isolated from the surrounding tissues, and an electrical stimulator (S-48, Grass Instruments, Quincy, Massachusetts, USA) was used to produce the twitch impulses. Stimulation was increased until a supramaximal response was achieved. That is, the intensity (voltage) of the impulses was increased progressively until the maximum gastric pressure reached a plateau despite subsequent increases in the voltage. This voltage was then increased a further 25% and all stimuli were applied at that intensity (approximately 10 V, 30 Hz, 0.25 ms), always at the end of expiration (determined using the esophageal pressure curve) and, when required, against the occluded airway. At least 3 stimuli were applied after each series of submaximal efforts. In order to minimize the number of animals in the study, the tests were performed twice under each experimental condition, with an interval of 15 minutes, as recommended by the local ethics committee.

**Diaphragmatic response.** The balloon catheters mentioned above were connected to pressure transducers (Validyne MP45-18, Northridge, California, USA), to measure the gastric (Pga) and esophageal (Pes) pressures. The transdiaphragmatic pressure (Pdi) was calculated automatically as the arithmetic difference between the two readings—Pga (usually positive) and Pes (usually negative)—(Figure 2).<sup>15</sup> However, the Pga induced by bilateral phrenic stimulation (the twitch Pga) was established as the principal variable of interest, given that the Pes induced in this way, and thus the resulting Pdi, could be directly affected by the stimulation.<sup>16</sup> Sonomicrometry was used to measure the initial length, the shortening (contraction), and the subsequent lengthening (relaxation) of each hemidiaphragm. For this purpose, during the laparotomy, 2 piezoelectric crystals were positioned on each costal hemidiaphragm, directly on the muscle fibers, separated by 10 to 13 mm. These crystals were connected to an analyzer (Triton Technology Inc, San Diego, California, USA). The length of the diaphragm at any given moment was expressed as a percentage of the initial length of the resting muscle (FRC, or length at functional residual capacity). The contractile speed was expressed as a percentage of the distance covered (in relation to the resting length) per second (FRC%/s).

The electromyographic response was also recorded using 2 copper electrodes, covered by a piece of polyester, placed on the left hemidiaphragm, 20 mm apart, sutured to the fascia via an abdominal approach, and with a conductive gel applied between the electrodes and the muscle. The data obtained were recorded on an 8-channel analog polygraph (HP 7758 B, Hewlett-Packard, Palo Alto, California, USA) and were then digitized (DT2821, Data Translation, Maryland, USA) and analyzed (Anadat-Labdat software, Rht-InfoDat, Montréal, Quebec, Canada).

**Additional recordings.** To ensure the animals remained stable throughout the procedures, oxygen saturation (tongue sensor), end-tidal carbon dioxide tension, and blood pressure were monitored at all times. These data were recorded using the above-mentioned analog polygraph. In addition, arterial blood samples were taken in 3 animals for serial determination of the PaO<sub>2</sub>, PaCO<sub>2</sub>, and pH, among other parameters.

**Subarachnoid anesthesia.** After carrying out the different maneuvers with spontaneous contribution of the different inspiratory muscles, spinal anesthesia was performed in order

TABLE 2  
**Pressures and Muscle Shortening Achieved by Supramaximal Bilateral Phrenic Stimulation Before and After the Different Series of Occlusive Efforts, Before and After Spinal Anesthesia<sup>a</sup>**

Before Anesthesia						
	Short Series		Medium Series		Long Series	
	Pre	Post	Pre	Post	Pre	Post
Pes, cm H <sub>2</sub> O	-11.8 (1.5)	-11.2 (1.6)	-11.7 (1.6)	-13.1 (1.7)	-11.6 (2.6)	-11.7 (3.1)
Pga, cm H <sub>2</sub> O	3.2 (0.4)	3.7 (0.3) <sup>b</sup>	3.5 (0.3)	3.9 (0.3) <sup>b</sup>	3.2 (0.4)	2.5 (0.3) <sup>c</sup>
Pdi, cm H <sub>2</sub> O	15.0 (1.7)	14.9 (1.8)	15.2 (1.6)	17.2 (1.8) <sup>b</sup>	14.7 (2.9)	14.2 (3.3)
Shortening						
RHD, ↓FRC%	48 (6)	44 (5)	47 (5)	53 (5) <sup>b</sup>	45 (7)	46 (7)
LHD, ↓FRC%	52 (7)	52 (8)	47 (6)	54 (6) <sup>b</sup>	37 (1)	35 (1) <sup>c</sup>
After Anesthesia						
	Short Series		Medium Series		Long Series	
	Pre	Post	Pre	Post	Pre	Post
Pes, cm H <sub>2</sub> O	-11.2 (1.5)	-10.6 (1.3)	-11.4 (1.4)	-12.6 (1.4)	-12.2 (1.5)	-12.2 (1.7)
Pga, cm H <sub>2</sub> O	3.6 (0.3)	4.0 (0.4) <sup>b</sup>	3.8 (0.2)	4.6 (0.2) <sup>c</sup>	4.0 (0.2)	3.6 (0.3) <sup>c</sup>
Pdi, cm H <sub>2</sub> O	14.8 (1.5)	14.6 (1.2)	15.2 (1.3)	17.2 (1.4) <sup>b</sup>	16.2 (1.2)	15.8 (1.7)
Shortening						
RHD, ↓FRC%	53 (8)	50 (7)	50 (7)	56 (7) <sup>b</sup>	41 (7)	42 (6)
LHD, ↓FRC%	38 (5)	37 (5)	42 (6)	46 (7)	43 (7)	44 (8)

Abbreviations: ↓FRC%, percentage of the initial length of muscle between the 2 piezoelectric crystals at functional residual capacity; LHD, left hemidiaphragm; RHD, right hemidiaphragm; Pdi, Pes, Pga, esophageal, transdiaphragmatic, and gastric pressures, respectively, induced by bilateral phrenic stimulation.

<sup>a</sup>Values are expressed as mean (SD).

<sup>b</sup>P<.005.

<sup>c</sup>P<.01.

to minimize the contribution of the intercostal muscles. To do this, 1 mL of a solution of tetracaine (Sigma-Aldrich, St Louis, Missouri, USA) was injected into the subarachnoid space in the lumbar region, with the head of the animal slightly raised. The efficacy of this procedure to abolish the electromyographic signal from the intercostal muscles was demonstrated in another study performed in parallel in the same laboratory.<sup>14</sup> Thirty minutes

later, the respiratory efforts were repeated and diaphragmatic activity was re-evaluated.

**Occlusions.** After determining the basal response level, series were performed with the airway occluded, with spontaneous inspiratory efforts by the animals. Short (5 efforts), medium (10 efforts), and long (20 efforts) series were performed, always

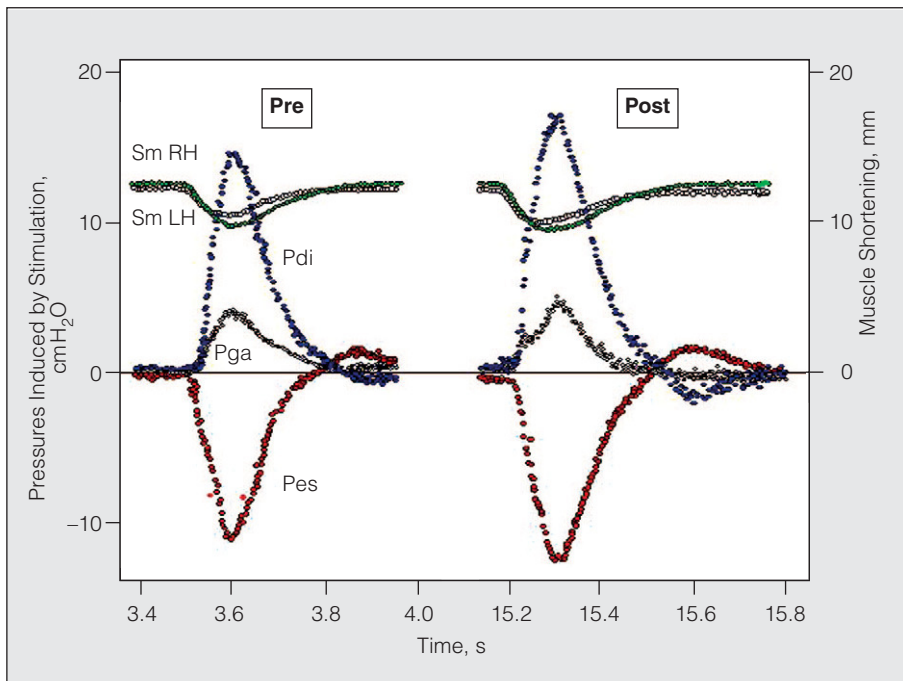


Figure 3. Changes in the respiratory pressures (gastric and esophageal pressures and transdiaphragmatic pressure calculated from the 2 previous signals) and muscle shortening induced by supramaximal twitch stimulation before and after the medium series of isometric inspiratory efforts with the airway occluded and, in this case, after subarachnoid anesthesia. For details, see text. Pdi indicates transdiaphragmatic pressure; Pes, esophageal pressure; Pga, gastric pressure; Sm LH, sonomicrometry of the left hemidiaphragm; Sm RH, sonomicrometry of the right hemidiaphragm.

allowing the animals to breathe again with the airway open for at least 4 cycles after each series. The hypothesis was that the ventilatory efforts against the occluded airway would approach a maximum level by the third or fourth occlusion (response plateau), and that this would occur with the spontaneous contribution of the different inspiratory muscles before spinal anesthesia, and without the participation of the intercostal muscles after this anesthesia. Measurement of the diaphragmatic response to electric stimulation was repeated after each series of efforts.

*Potentiation Protocol*

Bilateral diaphragmatic stimulation was performed immediately after the short, medium, and long series (in that order to minimize the cumulative fatigue effect), with an interval of at least 30 minutes between each series; measurements were taken before and after spinal anesthesia. As has already been stated, at least 3 stimuli were applied each time, and all tests were performed in duplicate.

*Statistical Analysis*

All measurements are expressed as mean (SD). Analysis of variance for repeated measures was used to compare each variable after the different series, and the Spearman correlation coefficient to estimate the degree of correlation between quantitative variables. Significance was established as  $P \leq .05$ .

**Results**

Table 2 shows the baseline values of the principal study variables and the results obtained after the different series of occlusive maneuvers. The results are given both before spinal anesthesia (all respiratory muscles active) and after its administration (abolition of the contribution of the intercostal muscles to ventilation).

*Baseline Values*

*Respiratory pressures before spinal anesthesia.* Pga and Pdi showed no significant variations between the different series, although the baseline Pga tended to be slightly higher before the medium series in comparison with the short series. This trend was not observed in comparison of the baseline Pga before the long series and the other values.

*Muscle shortening before spinal anesthesia.* No significant differences were observed between the baseline contraction of the right hemidiaphragm before the 3 series. However, on the left, there was slightly less baseline contraction before the long series with respect to the short and medium series ( $P < .01$ ).

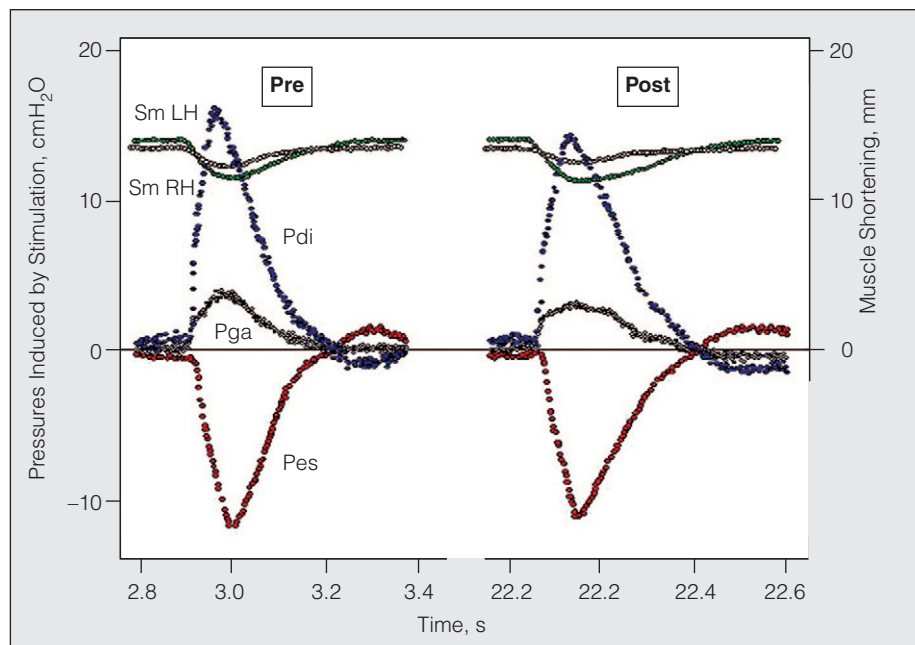
*Respiratory pressures after spinal anesthesia.* Baseline Pga values also showed a nonsignificant tendency to be higher before the medium series than before the short one and were significantly higher in the long series in comparison with the short one ( $P < .05$ ).

*Muscle shortening after spinal anesthesia.* The right hemidiaphragm showed less shortening before the long series ( $P < .01$ ).

*Effects of Occlusive Efforts on the Response*

*Respiratory pressures before spinal anesthesia* (Table 2A, before anesthesia). A small but significant increase was observed in Pga after the short series, but this was not seen in the other variables evaluated. Potentiation was more evident after the medium series, and the increase in Pga was associated with increases in Pes (borderline significance)

Figure 4. Graph of the respiratory pressures (gastric and esophageal pressures and transdiaphragmatic pressure calculated from the 2 previous signals) and diaphragmatic contraction-relaxation cycle induced by supramaximal twitch stimulation, before and after the long series of isometric inspiratory efforts with the airway occluded, and after the administration of subarachnoid anesthesia. Pdi indicates transdiaphragmatic pressure; Pes, esophageal pressure; Pga, gastric pressure; Sm LH, sonomicrometry of the left hemidiaphragm; Sm RH, sonomicrometry of the right hemidiaphragm.



and Pdi. In contrast, the long series produced a decrease in the contractile response, with a fall in Pga (not reflected in Pdi as a consequence of the stability of Pes).

*Muscle shortening before spinal anesthesia.* The short series did not produce any relevant changes in contractility. However, greater degrees of shortening were observed in both hemidiaphragms after the medium series. The speed of contraction also decreased slightly in this series (from 3.2 [0.4]  $\downarrow$ FRC%/s to 2.9 [0.2]  $\downarrow$ FRC%/s in the left costal diaphragm and from 3.1 [0.3]  $\downarrow$ FRC%/s to 2.8 [0.3]  $\downarrow$ FRC%/s in the right costal diaphragm,  $P < .05$  in both cases). The left diaphragm showed less shortening after the long series. In addition, the electromyographic signal of the left diaphragm revealed a certain degree of fatigue (150% increase in the square root of the mean of the signal amplitude). The speed of contraction showed a slight tendency to decrease (from 3.2 [0.3]  $\downarrow$ FRC%/s to 3.0 [0.2]  $\downarrow$ FRC%/s, not significant).

*Respiratory pressures after spinal anesthesia* (Table 2, after anesthesia). As observed before anesthesia, only Pga increased in both the short and medium series (Figure 3). There was also an increase in Pes of borderline significance and in Pdi after the medium series. In contrast, the long series once again showed a significant fall in Pga, with no effect on the other pressures (Figure 4).

*Muscle shortening after spinal anesthesia.* The contraction of both hemidiaphragms was somewhat greater after all the series than at baseline, but this never reached significance (borderline significance on the right). However, the speed of muscle shortening did become slower after the medium series (from 3.0 [0.2]  $\downarrow$ FRC%/s to 2.8 [0.2]  $\downarrow$ FRC%/s on the left, and from 3.1 [0.2]  $\downarrow$ FRC%/s to 2.9 [0.2]  $\downarrow$ FRC%/s on the right;  $P < .05$  in both cases), with no changes in the other series.

No correlations of interest were observed between the different variables, and the respiratory gases remained stable and within normal ranges throughout the study.

## Discussion

This study, performed on a model of animals under sedation, confirms that the strength of the diaphragm is modified by previous ventilatory effort. In addition, the use of sonomicrometry has shown that this change of pressure is associated with alterations of both the magnitude and speed of muscle contraction. The end result is a possibly greater or lesser response of the muscle to a given stimulus, depending on whether potentiation or fatigue predominates.

Determination of the maximum strength of the diaphragm or of all the respiratory muscles together, whether through voluntary maneuvers or by stimulation, has become increasingly relevant to clinical practice. However, the influence of certain ever-present factors on the response observed is often underestimated. Particularly important among these factors is the lung volume at which the response is measured, the possible occurrence of strong contractions before a measurement that is taken as valid, and changes in abdominal compliance.<sup>16-18</sup> In the present study, we have

investigated the influence of the recent contractile history of the diaphragm on its maximal response. We used bilateral electrical stimulation of the phrenic nerves, a useful method for the evaluation of muscle function, and one that is employed both in clinical practice and in experimental research.<sup>19,20</sup> Its main advantages derive from the fact that stimulation reaches the whole diaphragm and that it does not require the subject or experimental animal's collaboration. In addition to measuring the pressures, already done in previous studies,<sup>9,16</sup> we also performed sonomicrometry, which enabled us to evaluate the characteristics of the contraction itself. Furthermore, we used 3 patterns of previous activity to evaluate whether and to what extent these factors affect the response.

Potentiation, stepped response or, to use the term now preferred, postactivation potentiation, is a phenomenon through which previous muscle activity produces a greater contractile response in maximal maneuvers.<sup>16,21</sup> Response intensity increases progressively until it reaches a plateau of maximum potentiation, while contraction and relaxation speeds decrease.<sup>22</sup> This phenomenon occurs whether the previous contractions are voluntary or induced,<sup>22</sup> and it is more intense after isometric than dynamic contractions.<sup>23</sup> It is more marked in men than in women, decreases with age, and increases with strength training.<sup>23</sup> It has been described in limb muscles, but has also been observed in the diaphragm.<sup>9,18</sup> The intensity of potentiation can be very large (up to 50%-60%) and persist from about 10 seconds after completing the previous contractions to up to 5-20 minutes later.<sup>9</sup> Some authors suggest that it depends mainly on the intensity of the previous contractions.<sup>9</sup> Potentiation has been attributed to the slow return of calcium into the cisterns of the sarcoplasmic reticulum after its release with the stimulus. In addition, a specific factor is believed to be relevant in the case of the diaphragm, namely the progressively lower resistance to deformity of the thoracic cage after repeated ventilatory efforts.<sup>18</sup>

Postactivation potentiation should not be confused with so-called facilitation, which is an increase in muscle response if the stimulation occurs after a voluntary contraction has initiated.<sup>24</sup> In this case, it is thought that the residual calcium not released during the first part of the contraction, is released on receiving a second impulse, with a greater release of neurotransmitters (principally acetylcholine).<sup>25</sup> However, the specific response to the secondary stimulus is smaller than that of the primary stimulus. The explanation is that the stimulation can only recruit a few additional motor units.<sup>19</sup> Both potentiation and facilitation have been observed in the diaphragm.<sup>24</sup>

Fatigue is a phenomenon through which the muscle loses contractile efficacy for a certain time. Peripheral fatigue, or fatigue of the muscle itself, is classified according to the reduction in its response to high frequency (50-100 Hz) or low frequency (5-30 Hz) stimulation,<sup>26</sup> the latter occurring after spontaneous contractile activity of physiologic intensity.<sup>27</sup> It is associated with a depletion of muscle energy elements, such as high-energy phosphates and glycogen, or with the appearance of muscle microlesions. Recovery is therefore relatively slow. Although the fatigue phenomenon is common to all muscles

of the body, the diaphragm has specific metabolic characteristics, as its contraction depends to a great extent on extracellular calcium.<sup>28</sup> It is now believed that repeated, intense activity could deplete the calcium in the sarcoplasmic reticulum of the fibers.<sup>29</sup> Furthermore, a decrease has been observed in the diaphragmatic response to stimulation after a period of intense contractions on breathing against a resistive load<sup>30</sup>: this is related to the onset of acute fatigue. Fatigue of the diaphragm, particularly the low frequency type, can persist for 30 minutes to 1 hour after termination of the stimulus.<sup>30</sup> Potentiation thus disappears more rapidly than fatigue,<sup>9</sup> although the two phenomena can coexist.<sup>31,32</sup>

It should be noted that not only pressures but also the degree of muscle shortening were recorded in the present study. This was a novel aspect of the design. Moreover, in our model, the aim of the different series was not only to increase the number of efforts but also particularly to achieve greater pressures.<sup>33</sup> In the short series, potentiation was mild and only observed in the change in Pga, whereas in the medium series a trend could also be seen in the Pes and in an increase in the Pdi, as well as in the more marked and slower muscle shortening. This potentiation phenomenon becomes more marked after subarachnoid anesthesia (although it was still of moderate magnitude). This finding could be related to a greater previous exertion by the diaphragm, which had lost the added effect of the intercostal muscles. The authors of a previous study used a short series of efforts similar to ours and were unable to demonstrate potentiation, probably because of the low intensity of the stimulus in that study.<sup>21</sup>

With regard to the long series, the twitch Pga revealed a decrease in muscle response both before and after subarachnoid anesthesia. We believe that under both conditions there is a certain degree of fatigue together with the potentiation.<sup>9,21,34,35</sup> From a mechanical point of view, the pressures generated by the animals at the end of the long series were lower than the initial pressures; together with the changes in the electromyographic signal, this would support the possibility of combined potentiation and fatigue. It is known that fatigue reduces the diaphragmatic response to twitch stimulation,<sup>30</sup> and the existence of this phenomenon with or without a certain degree of potentiation, could therefore lead to slight increases, stability, or even decreases in the contractile response. The final result would depend on the relative impact of each factor at the time the measurement was taken. In our case, the combination of factors brought about a slight decrease in Pga, with minimal detectable repercussion on other pressures or on muscle shortening.

Previous studies have underlined the importance of achieving reliable maximum respiratory pressures both in healthy individuals and in patients. For some authors, determination of the static maximum inspiratory pressure measured at the mouth has acceptable reproducibility<sup>36,37</sup> and, in addition, would lack any significant learning effect,<sup>38</sup> in contrast to forced inhalation,<sup>39</sup> which would require at least 6 previous attempts before achieving what may be considered the maximum pressure.<sup>38</sup> However, for other authors such as Fiz et al,<sup>7</sup> a minimum of 9 measurements are required to achieve a reproducible, peak maximum

inspiratory pressure, particularly in patients with chronic obstructive pulmonary disease, as reproducibility does not necessarily imply validity of the maximum value.<sup>39</sup> The problem of the need for so much repetition of maneuvers can be attenuated by first performing maneuvers of a lower intensity,<sup>8</sup> though this probably means that the improvement could be due to potentiation. In another study, the same authors demonstrated the onset of relatively persistent inspiratory fatigue (30 minutes) with a fall in the maximum inspiratory pressure after breathing against increased resistances.<sup>40</sup> With respect to the maximum pressures induced by stimulation in humans, it is generally accepted that their reproducibility is similar to that of maneuvers of forced inhalation (sniff), and that they are sufficient for clinical purposes.<sup>6,37</sup>

The findings of the present study have a number of implications. Although the techniques usually used to measure maximum respiratory pressures are not called into question, our study does draw attention to the conditions under which such pressures should be measured, with specific reference to previous activity of the respiratory muscles, as this can lead to an increase or decrease in the contractile response. In other words, the spontaneous activity of the muscle must first be taken into account, and the accuracy of measurements performed under conditions of clinical instability, in which potentiation and fatigue can exist in isolation or in combination, must be questioned. For example, the 2 phenomena may coexist in patients in whom weaning from mechanical ventilation is being contemplated. In patients who are unable to collaborate, both phenomena may develop after repeated efforts against the occluded airway, a technique used to determine their maximum inspiratory force; this can also occur in collaborating patients after a major ventilatory effort (eg, after a test with a T-tube). The difficulty in the first case is to establish the appropriate number of occlusive efforts to obtain a valid response without potentiation or fatigue and, in the second, is to determine which of the 2 factors predominates at any given moment, complicating comparisons with other measurements in the same subject.

Functional determination of the maximum respiratory pressures in patients with stable disease is another situation that must be considered. Here we must take into account the exertion caused by maneuvers prior to the one considered valid, as we have seen that there is a degree of potentiation even after only a few efforts. If maneuvers must be repeated too many times in order to obtain a valid value, it would probably be preferable to allow a reasonable time to pass and start again.

A possible limitation of our model is that it was performed in laparotomized animals and under sedation. After closure by tissue planes, it is unlikely that the surgical wound would have any relevant effect on the results. In any case, this situation was identical under all conditions and is common in this type of study.<sup>12-14,33,41</sup> Pentobarbital is known to have the potential to reduce respiratory muscle activity to some degree.<sup>42</sup> However, it may be considered that this effect is minor,<sup>43</sup> does not modify the relative contribution of the different respiratory muscles,<sup>43</sup> and is much smaller than the effect of alternative inhaled anesthetics.<sup>44</sup> For this reason, the type of sedation used in

the present study has been used extensively in the investigation of muscle mechanics.<sup>12-14,44,45</sup> In addition, care was taken to ensure that sedation was stable during all the experimental conditions in which the stiffness of the abdominal wall was modified.

Despite our observations, we must consider that the measurement of respiratory pressures is a relatively stable method in a single individual, both for the variables obtained through voluntary maneuvers and for those derived from stimulation.<sup>6,18,19,37</sup> However, without casting doubt on the clinical utility the results obtained in such studies, we argue that in order to better evaluate them, it is necessary to take into consideration the factors we have investigated (potentiation and fatigue).

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