Effects of Acute Respiratory and Metabolic Acidosis on Diaphragm Muscle Obtained from Rats

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ABSTRACT

Background: Acute respiratory acidosis is associated with alterations in diaphragm performance. The authors compared the effects of respiratory acidosis and metabolic acidosis in the rat diaphragm *in vitro*.

Methods: Diaphragmatic strips were stimulated *in vitro*, and mechanical and energetic variables were measured, cross-bridge kinetics calculated, and the effects of fatigue evaluated. An extracellular pH of 7.00 was obtained by increasing carbon dioxide tension (from 25 to 104 mmHg) in the respiratory acidosis group (n = 12) or lowering bicarbonate concentration (from 24.5 to 5.5 mM) in the metabolic acidosis group (n = 12) and the results compared with a control group (n = 12, pH = 7.40) after 20-min exposure.

Results: Respiratory acidosis induced a significant decrease in maximum shortening velocity (–33%, $P < 0.001$), active isometric force (–36%, $P < 0.001$), and peak power output (–59%, $P < 0.001$), slowed relaxation, and decreased the number of cross-bridges (–35%, $P < 0.001$) but not the force per cross-bridge, and impaired recovery from fatigue. Respiratory acidosis impaired more relaxation than contraction, as shown by impairment in contraction–relaxation coupling under isotonic (–26%, $P < 0.001$) or isometric (–44%, $P < 0.001$) conditions. In contrast, no significant differences in diaphragmatic contraction, relaxation, or contraction–relaxation coupling were observed in the metabolic acidosis group.

Conclusions: In rat diaphragm, acute (20 min) respiratory acidosis induced a marked decrease in the diaphragm contractility, which was not observed in metabolic acidosis. (ANESTHESIOLOGY 2015; 122:876-83)

HYPERCAPNIA induces respiratory acidosis and may occur with any cause of acute respiratory failure. It also frequently occurs in mechanically ventilated patients particularly because the use of low tidal volumes is now widely recommended (permissive hypercapnia).[1] The effects of acute respiratory acidosis have been well documented on hemodynamics and pulmonary function and, more recently, on inflammatory and infectious processes.[2] However, its effects on diaphragmatic function may have important consequences, particularly during noninvasive ventilation or during the weaning process. It has been recently documented that the recovery of diaphragmatic contractility after a period of hypercapnia may be delayed.[3]

Several *in vivo* studies have shown that acute respiratory acidosis alters diaphragmatic contractile force both in animals models[4,5] and in humans.[6,7] However, the precise mechanisms of diaphragm weakness remain a matter of debate because several indirect mechanisms may be involved, such as complex changes in neuroventilatory drive and modification of the length–force relationship of the diaphragm because of variations in pulmonary volume, precluding a precise analysis of respiratory muscle function *in vivo*. Yanos *et al.*[5]

What We Already Know about This Topic

- Diaphragm function is altered in acidosis, but whether the source of acidosis causes alterations in function is not known.

What This Article Tells Us That Is New

- Diaphragm strips were exposed to a pH of 7.0 either using carbon dioxide or to strips exposed to low bicarbonate and both compared with strips exposed to a pH of 7.4. The strips exposed to high carbon dioxide had impairment in contraction and even more impairment in relaxation–contraction coupling. In contrast, diaphragmatic strips exposed to lower bicarbonate had no significant differences in diaphragmatic contraction, relaxation, or contraction–relaxation coupling.

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observed that diaphragmatic performance was depressed by acute respiratory acidosis but not by lactic acidosis *in vivo* in the dog, but they could not rule out indirect effects of lactic acidosis (catecholamines, hemodynamics, lactates). A few *in vitro* studies have directly assessed the effects of acute respiratory acidosis on diaphragmatic muscle and have confirmed a decrease in intrinsic contractility.[8] However, it has not been possible to delineate the effects related to carbon dioxide and those of pH itself. Lactic acidosis was shown

to decrease diaphragmatic contractility only at very low extraphysiological pH (6.80).[9] Moreover, there are some controversies concerning intracellular pH changes induced by metabolic acidosis.[10–12] We recently demonstrated that acute respiratory acidosis markedly decreases intracellular intracellular pH, whereas metabolic acidosis does not in isolated cardiomyocytes.[13]

Thus, we examined diaphragmatic functional modifications induced by acute respiratory acidosis in the rat *in vitro*, under baseline condition and during fatigue, and compared them with those induced by comparable metabolic acidosis. We hypothetized that respiratory acidosis impairs diaphragmatic force more than metabolic acidosis. Our experimental model also enabled us to precisely assess the effects of acidosis on mechanics, including relaxation and contraction–relaxation coupling, energetics, and cross-bridges kinetics.[14,15]

Materials and Methods

Care of the animals conformed to the officials recommendations of the French Ministry of Agriculture (Paris, France) and the Helsinki Declaration. Thus, these experiments were conducted in authorized laboratories and under the supervision of authorized researchers. Six-week-old male Wistar rats (Charles River Laboratories, L'Abresle, France) were maintained on a 12:12-h light dark photoperiod receiving rat chow and water *ad libitum* and were assigned to a control group, a respiratory acidosis group, or a metabolic acidosis group.

Mechanical and Energetic Parameters

After a brief anesthesia with thiopental, rats were killed and a muscle strip from the ventral costal diaphragm was dissected from the muscle *in situ*. This diaphragm strip was immediately vertically suspended in a 200-ml jacketed reservoir with Krebs–Henseleit bicarbonate buffer solution (NaCl 118 mM, KCl 4.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.1 mM, $NaHCO_3$ 25 mM, $CaCl_2$ 2.5 mM, and glucose 4.5 mM) prepared daily with highly purified water. The bathing solution was bubbled with 95% oxygen and 5% carbon dioxide, resulting in a pH 7.40 and maintained at 29°C.[14–16]

Preparations were field-stimulated (30 V) by using two platinum electrodes with rectangular wave pulses of 1 ms duration at 10 pulses/min in the twitch mode. After a 30-min stabilization period, at the apex of the length-active isometric force curve (L_{max}), diaphragm muscle strips recovered their optimal mechanical performance.[17,18] Measurements of mechanical variables were made at L_{max} under tetanic stimulation at 50 Hz (10 trains each minute of 300 ms duration, with 1 ms rectangular pulses) to obtain maximal contractions. Cross-sectional area was calculated from the ratio of muscle weight to muscle length, assuming a muscle density of 1.06.

The electromagnetic lever system has been described previously.[19] All the analyses were made from digital records of force and length obtained using specific software, as previously described.[14,20] Contraction variables including the maximum shortening velocity (V_{max}), the extent of shortening (ΔL), peak of the positive derivative normalized to cross-sectional area (+dF/dt), and the active force normalized to cross-sectional area (AF) were measured. Variables characterizing relaxation including the maximum lengthening velocity (Vr_{max}) and peak of the negative derivative normalized per cross-sectional area (–dF/dt) were also determined. Mechanical variables were calculated from three consecutive tetanic contractions preloaded at L_{max} with increasing afterload from zero load to fully isometric contraction. The first contraction was abruptly clamped to zero load just after the electrical stimulus, with critical damping to slow the first and rapid shortening overshoot resulting from the recoil of series passive elastic components, enabling determination of V_{max}. The second contraction was isotonic and loaded with preload only. ΔL and Vr_{max} were determined from this contraction. The last contraction was fully isometric at L_{max}. AF, +dF/dt, and –dF/dt were determined from this fully isometric contraction. Because changes in the contraction phase induce coordinated changes in the relaxation phase, relaxation parameters cannot directly assess lusitropy. Therefore, we calculated the ratios $Vr_{max}/\Delta L$ and $-dF/dt/AF$ that assessed lusitropy in isotonic and isometric conditions.[16]

The force–velocity curve was derived from the peak shortening velocity plotted against the total force (resting force + active force) normalized for cross-sectional area.[20] Both measurements were obtained from 10 tetanic contractions, from zero load to fully isometric contraction. The total force–velocity curve was fitted according to the Hill equation.[21] The following energetic parameters were derived from the Hill hyperbola: the curvature of the hyperbola (G), nonnormalized maximum power output (E_{max}), and maximum mechanical efficiency (Eff_{max}). The following variables of cross-bridge kinetics were determined from mechanical data with the equations of Huxley[22]: the total number of cross-bridges per mm^2 (m), elementary force per cross-bridge (π), the maximum value of the rate constant for cross-bridge attachment f1, the maximum values of the rate constants of cross-bridge detachment g1 and g2, and the time cycle (tc) that reflects myosin adenosine triphosphatase activity.[15,16]

Metabolic and Respiratory Acidosis

A separate 200-ml jacketed reservoir with the same Krebs–Henseleit bicarbonate buffer solution was prepared. In the respiratory acidosis group, the bathing solution was bubbled with two gases (95% oxygen-5% carbon dioxide and 50% oxygen-50% carbon dioxide). The proportions of these two gases were adjusted until a stable pH of 7.00 was obtained, the solution being maintained at 29°C. In the metabolic acidosis group, the bathing solution was initially prepared without bicarbonate, and then sodium bicarbonate was added until a pH of 7.00 was obtained. Final concentrations of sodium and calcium were adjusted. Electrolyte concentrations were measured with standard electrodes (Ektachem 500, Johnson

& Johnson, Les Ulis, France). Oxygen and carbon dioxide tensions and pH were measured with standard electrodes at 37°C (IL-BG3, Instrumentation Laboratory, Saint Mandé, France). The new solution (metabolic or respiratory acidosis) was rapidly switched, and the effects were recorded every 5 min over 20 min. Blinding was not feasible, and no randomization was performed for group allocation.

Effect of Fatigue

After determination of baseline values, fatigue was induced by repeatedly stimulating the diaphragmatic strip with 75 trains/min of 300 ms duration at a stimulation frequency of 50 Hz. Stimulation continued until the muscle strip was fatigued to a point where it generated 65% of its original tetanic tension measured before the fatigue procedure. Mechanical variables were recorded just after completion of the fatigue procedure and after 20 min of recovery.

Statistical Analysis

Data are expressed as means ± SD. Comparisons between groups were performed using repeated measure analysis of variance and Newman–Keuls test. The total force–velocity relationship was fitted to a hyperbola using multilinear regression and the least square method.[15,16] Assuming a baseline value of AF of 170 ± 15 mN/mm^2, an α risk of 0.05, and a β risk of 0.20, we determined that a sample size of n = 12 per group would enable us to detect a 10% decrease in AF (PASS 11 software, Statistical Solutions Ltd., Cork, Ireland). All P values were two-tailed, and a value of $P < 0.05$ was considered significant. Statistical analysis was performed using NCSS 2007 software (Statistical Solutions Ltd.).

Results

Body weight was 328 ± 10 g, L_{max} was 16 ± 1 mm, and resting force 11 ± 1 mN. No significant differences between groups were observed in baseline conditions. A mean pH of 7.00 was obtained in the two acidosis groups, associated with a high value of carbon dioxide in the respiratory acidosis group and a low bicarbonate value in the metabolic acidosis group (table 1).

Mechanical and Energetic Properties

No significant differences in baseline values of mechanical and energetic parameters were observed between the three groups (table 2). In the acute respiratory acidosis group, we observed a significant decrease in ΔL (–15%), V_{max} (–33%), AF (–36%), and +dF/dt (–62%). The decrease in AF over the 20 min of exposure to acidosis is shown in figure 1. In the acute respiratory acidosis group, the decreases in relaxation parameters in isotonic (Vr_{max}, –26%) and isometric (–dF/dt, –70%) conditions were more pronounced, leading to a significant decrease in contraction–relaxation coupling parameters both in isotonic ($Vr_{max}/\Delta L$, –26%) and in isometric (–dF/dt/AF, –44%) conditions. In contrast, no significant differences were observed in the metabolic acidosis group (fig. 1, table 2).

In the acute respiratory acidosis group, the effects on force and velocity were responsible for a significant decrease in the peak power output (–59%) but without significant changes in the curvature of the force–velocity curve (table 2, fig. 1). In contrast, no significant differences were observed in the metabolic acidosis group (table 2, fig. 2).

In an additional group (n = 7), we studied the recovery after exposure to respiratory acidosis. In this group, respiratory acidosis also induced a marked decrease in AF ($58 \pm 10\%$ of baseline, $P < 0.001$), but recovery was not complete after return to normal pH ($82 \pm 9\%$ of baseline, $P < 0.001$).

Cross-bridge Kinetics

No significant differences in baseline values of cross-bridge kinetics were observed between the three groups (table 2). In the respiratory acidosis group, the number of cross-bridges (m) was significantly decreased (–35%), but the force per cross-bridge remained unchanged (table 2). Acute respiratory acidosis also induced step-dependent modifications of the cross-bridge kinetics, as shown by significant reduction in the maximum rate constant for cross-bridge detachment (g2, –31%) and in the maximum rate constant for cross-bridge attachment (f1, –37%). In addition, the overall duration of the cross-bridge cycle (tc) was significantly prolonged (+61%). In contrast, metabolic acidosis did not significantly modify cross-bridge kinetics.

Table 1. Comparison of Gases and Electrolytes in the Krebs–Henseleit Solution in the Three Experimental Groups

	Control (n = 12)	Metabolic Acidosis (n = 12)	Respiratory Acidosis (n = 12)
pH	7.39 ± 0.02	7.00 ± 0.02	7.00 ± 0.02
Po$_2$ (mmHg)	560 ± 40	571 ± 41	545 ± 43
Pco$_2$ (mmHg)	25 ± 1	25 ± 1	104 ± 6*†
Bicarbonates (mM)	24.5 ± 0.7	5.5 ± 0.2*	24.6 ± 0.7†
Sodium (mM)	151 ± 3	151 ± 2	151 ± 3
Potassium (mM)	5.4 ± 0.2	5.4 ± 0.2	5.3 ± 0.1
Calcium (mM)	2.51 ± 0.10	2.51 ± 0.08	2.52 ± 0.10

Values are expressed as mean ± SD. No statistical comparison was performed for pH.

*$P < 0.05$ *versus* control group. †$P < 0.05$ *versus* metabolic acidosis group.

Pco$_2$ = partial pressure of carbon dioxide; Po$_2$ = partial pressure of oxygen.

Table 2. Baseline Values of Mechanical and Energetic Parameters

	Control (n = 12)		Metabolic Acidosis (n = 12)		Respiratory Acidosis (n = 12)	
	Baseline	20 min	Baseline	20 min	Baseline	20 min
Mechanics						
ΔL (%)	27±1	27±1	28±2	27±2	27±2	23±5*
V_{max} (L_{max}/s)	5.5±0.7	5.3±0.8	5.8±0.9	4.9±0.8	5.8±0.9	3.9±0.8*
Vr_{max} (L_{max}/s)	8.0±0.9	7.6±1.0	8.4±1.8	8.0±1.9	8.5±1.5	6.3±1.1*
$Vr_{max}/\Delta L$ ($L_{max} \cdot s^{-1} \cdot \%^{-1}$)	0.29±0.02	0.28±0.03	0.30±0.04	0.29±0.04	0.31±0.04	0.26±0.02*
AF (mN/mm^2)	169±17	169±18	165±16	160±21	172±13	110±25*
+dF/dt (mN · mm^{-2} · s^{-1})	2225±560	1946±823	2178±391	2016±442	2226±382	839±239*
−dF/dt (mN · mm^{-2} · s^{-1})	4591±962	4868±850	4140±1098	4071±1141	4660±976	1421±906*
−dF/dt/AF (mm^{-2} · s^{-1})	26±4	28±3	25±5	25±5	27±5	15±2*
Energetic						
G	4.7±1.7	4.7±2.1	4.5±1.8	3.9±2.1	4.5±1.3	4.9±2.4
E_{max} (mN · mm^{-2} · s^{-1})	44±11	47±18	51±17	47±14	46±15	19±8*
Eff_{max} (%)	45±12	46±11	46±12	41±14	47±11	47±12
Cross-bridge kinetics						
m (10^9/mm^2)	19±2	20±3	19±3	20±4	20±2	13±3*
π (pN)	8.8±0.6	8.7±0.9	8.8±0.7	8.2±1.2	8.6±0.8	8.6±0.7
tc (s)	0.32±0.17	0.31±0.25	0.35±0.24	0.31±0.21	0.28±0.19	0.45±0.42*
g1 (s^{-1})	35±23	36±32	39±34	46±28	39±22	32±19
g2 (s^{-1})	978±136	972±146	1063±177	977±154	1050±160	725±152*
f1 (s^{-1})	157±45	170±74	166±57	187±74	199±113	125±38*

Values are expressed as mean ± SD.

*$P < 0.05$ *versus* baseline values.

AF = isometric active force normalized per cross sectional area (CSA); ΔL = maximum extent of shortening; +dF/dt = peak of positive force derivative normalized per CSA; −dF/dt = peak of negative force derivative normalized per CSA; Eff_{max} = maximum mechanical efficiency; E_{max} = non normalized maximum power output; f1 = the maximum value of the rate constant for cross-bridge attachment; G = curvature of the hyperbola; g1 and g2 = the maximum values of the rate constants of cross-bridge detachment; m = total number of cross-bridges per mm^2; π = elementary force developed per cross-bridge; tc = time cycle; V_{max} = maximal unloaded shortening velocity; Vr_{max} = maximum lengthening velocity.

Fatigue

After the fatigue protocol, a comparable value of AF was reached in each group. The recovery from fatigue was impaired in the acute respiratory acidosis group compared with the control group but not in the metabolic acidosis (fig. 3).
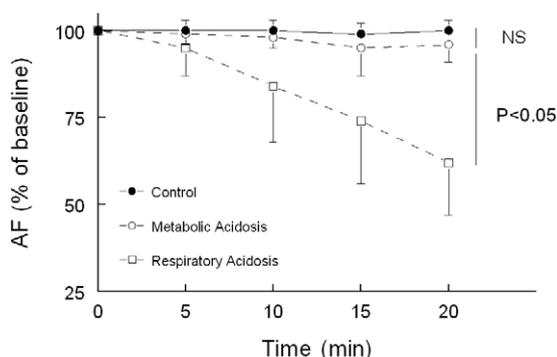

Fig. 1. Comparison of the time course of the effects of acute metabolic acidosis (pH =7.00, n = 12) and respiratory acidosis (pH = 7.00, n = 12) compared with a control group (pH = 7.40, n = 12) in isometric conditions. Data are mean percentage of baseline ± SD. AF = isometric active force for cross-sectional area; NS = not significant.

Discussion

We observed that acute respiratory acidosis significantly impaired the isolated diaphragm muscle taken from rats, mainly as a decrease in velocity of shortening, force, and peak power output, with more pronounced effect on relaxation (table 2), and an impaired recovery from fatigue (fig. 3). We found that acute respiratory acidosis significantly affected the number of active cross-bridge, as well as their kinetics, slowing the overall duration of the cross-bridge cycle (table 2). In contrast, no significant changes in diaphragm muscle performance were observed with metabolic acidosis. These results concords with our study showing that acute respiratory acidosis decreases intracellular pH, whereas metabolic acidosis does not.[13]

There are some controversies concerning diaphragmatic function in acute respiratory acidosis. A reduced diaphragmatic performance has been reported *in vivo*,[3–5,23] including in humans.[6,7] Nevertheless, Mador *et al.*[24] observed no significant effect of moderate hypercapnia (60 mmHg, arterial pH 7.27) in healthy volunteers. However, the mechanism of these changes remains a matter of debate. In such conditions, the combined effects of hypercapnia on pulmonary volume, nerve conduction, and neuroventilatory drive
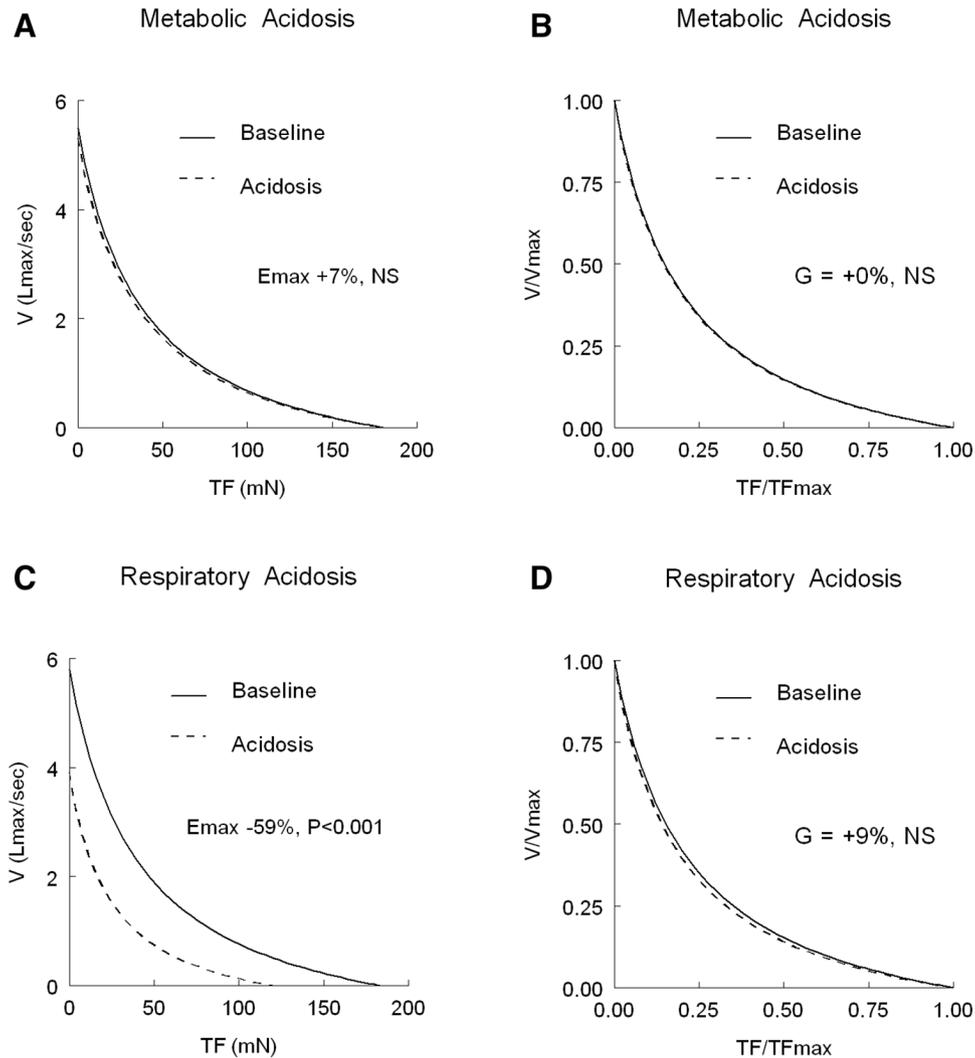
Fig. 2. Total force (TF)–velocity (V) curves in control conditions (pH = 7.40, n = 12), metabolic acidosis (pH = 7.00, n = 12), and respiratory acidosis (pH = 7.00, n = 12). (*A* and *C*) Absolute values were used to determine the peak power output (E_{max}). (*B* and *D*) The normalized TF and V values were used to determine the normalized peak power output and the curvature (G) of the TF-V curve. The curves are representative of a group and not a fit obtained from experimental data, meaning that we used in the equation of the curve the mean maximum force, maximum velocity, and curvature. Respiratory acidosis but not metabolic acidosis significantly decreased E_{max}. No significant changes occurred in G. NS = not significant.

preclude a precise analysis of respiratory muscle function *in vivo*. Few studies have investigated the intrinsic diaphragmatic mechanical properties. Using the hemidiaphragm preparation, Fitzgerald *et al.*[25] observed that both respiratory and metabolic acidosis decreased diaphragmatic force of contraction, but they tested very high, unphysiological, carbon dioxide concentrations (>180 mmHg, pH <6.90) and the experimental preparation used does not enable to rule out effects on the neuromuscular junction. However, Esau[8] showed that hypercapnia (84 mmHg, pH 7.11) decreases contractility and impaired recovery from fatigue in the hamster diaphragm. Our study showed that acute respiratory acidosis decreases diaphragmatic contractility, whereas metabolic acidosis does not, confirming the *in vivo* data obtained in the dog.[5] In our study, recovery from respiratory acidosis

was not complete in contrast to our results in cardiomyocytes.[13] However, this incomplete recovery in diaphragmatic strips concords with results obtained in the pig *in vivo* by Jaber *et al.*[3]

Although most experimental studies have concluded that acute respiratory acidosis induces a decrease in intracellular pH,[8,26] the effects of metabolic acidosis on intracellular pH have been debated. Adler *et al.*[27] found that intracellular pH tends to follow extracellular pH regardless of the method used to induce acidosis (hypercapnia or low bicarbonate concentration). Using ^{31}P nuclear magnetic resonance, Fitzgerald *et al.*[28] observed a significant decrease in intracellular pH in the rat hemidiaphragm when extracellular pH was lowered to 7.00. In contrast, using absorption spectrophotometry Zhang *et al.*[29] observed that intracellular pH is
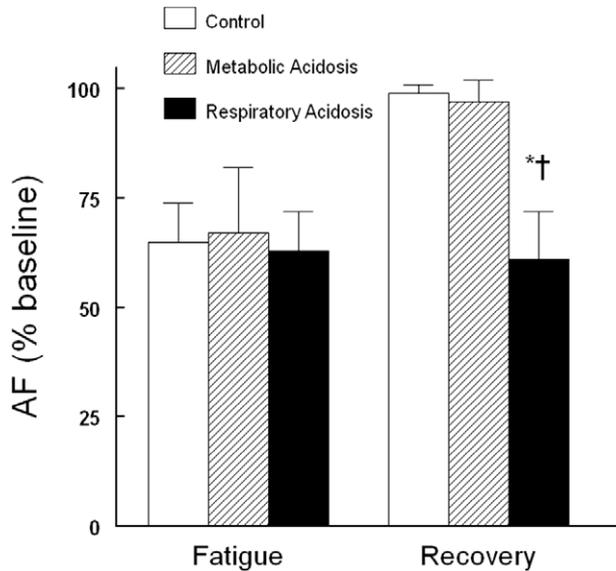
Fig. 3. Evolution of isometric active force (AF) normalized per cross-sectional area during fatigue and early recovery phase of fatigue control conditions (pH = 7.40, n = 12), metabolic acidosis (pH = 7.00, n = 12), and respiratory acidosis (pH = 7.00, n = 12). Values are expressed as mean percentage of baseline ± SD. *$P < 0.05$ *versus* control group; †$P < 0.05$ *versus* metabolic acidosis group.

stable over a wide range of extracellular pH (6.80 to 7.80) during acute respiratory acidosis. The discrepancies between these studies have not been resolved, but it should be pointed out that intracellular pH measurements were indirect in these previous studies. It should be also noted that the study from Adler *et al.*[27] suggests that different thresholds may exist for extracellular pH in respiratory acidosis and metabolic acidosis, a significant decrease in contraction being observed below an extracellular pH of 7.20 in respiratory acidosis and below a pH of 6.80 in metabolic acidosis. Heisler[26] also reported the existence of a plateau (extracellular pH 7.40 to 7.15) during which intracellular pH remains essentially constant, whatever the cause of acidosis. Using confocal microscopy and the fluorescent marker SNARF-1, we recently directly measured the intracellular pH in isolated cardiomyocytes and demonstrated that acute respiratory acidosis decreases intracellular pH, whereas metabolic acidosis does not.[14] Our results on diaphragmatic contraction are in agreement with these previous *in vitro* results[13] and also are in agreement with comparable *in vivo* studies.[9] It should be pointed out that metabolic blockade, ischemia (or hypoxia *in vitro*) are not the most appropriate means to study the intrinsic effect of acidosis because they induced numerous adverse effects mainly related to decrease in adenosine triphosphate production. The use of lactates may also not be optimal because it can bind calcium, thus indirectly decreasing contractility.[30]

Esau[8] did not observe significant effect of acute respiratory acidosis on relaxation (measured by the time constant of isometric relaxation), but no attempt was made

to measure contraction–relaxation coupling. We observed that the effects of acute respiratory acidosis on relaxation variables were more pronounced than those on contraction variables, reflecting an impairment in contraction–relaxation coupling. Relaxation is controlled by a complex interplay between loading conditions and inactivation, which is mainly limited by active calcium pumping by the sarcoplasmic reticulum, calcium removal from troponin C, and the instantaneous number of working cross-bridges.[31] However, there are marked physiological differences between isometric and isotonic relaxation. Up to 80% of maximum isometric force, passive mechanisms mainly determined isometric relaxation whereas, under fully isometric contraction, the calcium myofilament sensitivity seems to play a major role.[17] Thus, ours results suggest that acute respiratory acidosis reduces isometric relaxation through a modification of calcium myofilament sensitivity. The calcium affinity of troponin C is actually known to be pH sensitive.[32] Under isotonic conditions that correspond to important sarcomere shortening, the sensitivity of troponin C to calcium is low and the relaxation proceeds more rapidly and the calcium uptake by the sarcoplasmic reticulum seems to play a major role.[16] Thus, our results suggest that acute respiratory acidosis reduces isotonic relaxation through a decrease in calcium uptake by the sarcoplasmic reticulum. The reuptake of calcium by the sarcoplasmic reticulum is an active process, which requires enzymes that are actually known to be pH sensitive.[33] Acidosis may also increase membrane resistance and thus slow relaxation.[8] In patients experiencing respiratory muscle weakness, delayed relaxation may lead to incomplete relaxation, especially at high respiratory rates. Because diaphragmatic blood flow is impeded at a high transmural pressure, delayed or incomplete relaxation may facilitate diaphragmatic ischemia. Thus, impairment in diaphragmatic relaxation may increase the cost of breathing and promote diaphragmatic fatigue and respiratory failure.

The Huxley's cross-bridge original model was used to determine the effects of acidosis.[22] The π values calculated in our study (table 2) agree with those previously calculated in adult rat diaphragm[14,15] and are within the range of the force measured between actin filament and a single myosin molecule using optical tweezers.[34] We observed that acute respiratory acidosis decreased the number of cross-bridges but not the force per cross-bridge. Given that muscle performance depends on both the number of cross-bridges generating force and the unitary force generated during the power stroke, a reduced number of force-generating cross-bridges was a likely explanation for the depressed contractile performance in diaphragm muscle. Moreover, we observed that acute respiratory acidosis induced step-dependent modifications of the cross-bridge kinetics modifying f1 and g2, but not g1. The constant f1 corresponds to attachment step, whereas g1 and g2 correspond to the detachment step of actin from myosin.[13] The reduction in both f1 and g2 attested for longer duration for the cross-bridge attachment and detachment. Because the total duration of cross-bridge cycle (tc)

is strongly dependent on f1, it is not surprising that it was also modified by acute respiratory acidosis. The myosin adenosine triphosphatase responsible for tc value is actually pH sensitive.[35] However, our study does not enable us to separate the effects of carbon dioxide from those of pH on cross-bridges kinetics.

The following points should be considered when assessing the clinical relevance of our results. First, this *in vitro* study only dealt with intrinsic diaphragmatic contractility. Observed changes in diaphragmatic function *in vivo* in respiratory and metabolic acidosis depend also on modifications in central nervous system respiratory drive, neuromuscular transmission, and diaphragmatic arterial blood flow. It should be emphasized that, in clinical conditions, metabolic acidosis is most often related to shock that may induce marked alteration in cardiac output and diaphragmatic arterial blood flow, although the last one seems to be spared during hemorrhagic shock.[36] The inflammatory process associated with shock may also interfere with diaphragmatic function.[37] Second, this study was conducted at low temperature, at low-frequency stimulation, and at high oxygen pressure. However, diaphragmatic muscle must be studied at low temperature because stability of mechanical parameters is not sufficient at 37°C, and they must be studied at low frequency and high oxygen pressure because high-frequency stimulation induces core hypoxia. Nevertheless, respiratory acidosis might have some protective effects that can be modulated by hypoxemia (in comparison with normoxia), particularly when considering acute lung injury.[38] Third, this study was conducted in healthy and young animals, and thus the effects of acidosis observed cannot be extrapolated to aging diaphragm or diseased diaphragm.[14] Fourth, this study was performed in the rat, and species differences cannot be excluded although myosin heavy chain quota is quite similar in rat and human diaphragms.[39] Fifth, our results may not apply to lower values of extracellular pH[28] or other tissues, including skeletal muscles. There are some recognized cell type variations in the Δintracellular pH/Δextracellular pH ratio.[40] We also studied only one level of acidosis (pH = 7.00), and the duration of exposure was limited to 20 min and thus, we cannot exclude that more prolonged exposure to metabolic acidosis may have produced an effect. Lastly, we studied acute acidosis, and its effects may markedly differ from those induced by chronic acidosis. For example, moderate chronic hypercapnic acidosis has been shown to modify size and composition of diaphragmatic fibers[41] and protect against ventilator-induced diaphragmatic dysfunction.[42]

In conclusion, in rat diaphragm, acute (20 min) respiratory acidosis induced a marked decrease in the diaphragm contractility, which was not observed in metabolic acidosis.

Acknowledgments

Competing Interests

The authors declare no competing interests.

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