Intensive Care Med (2003) 29:103–110
DOI 10.1007/s00134-002-1548-3

EXPERIMENTAL

Xavier Capdevila
Sandrine Lopez
Nathalie Bernard
Emmanuel Rabischong
Michèle Ramonatxo
Guilhem Martinazzo
Christian Prefaut

Effects of controlled mechanical ventilation on respiratory muscle contractile properties in rabbits

Received: 10 January 2002
Accepted: 4 October 2002
Published online: 12 December 2002

This research was supported by the Association pour le Développement de la Recherche et de la Formation en Anesthésie Réanimation. Centre Hospitalier Universitaire de Montpellier, France

X. Capdevila (✉) · S. Lopez · N. Bernard
E. Rabischong · G. Martinazzo
Département d'Anesthésie Réanimation A,
Hôpital Lapeyronie et UPRES EA 701,
371 Avenue du doyen G Giraud,
34295 Montpellier, France
e-mail: x-capdevila@chu-montpellier.fr
Tel.: +33-467-338256
Fax: +33-467-337960

M. Ramonatxo · C. Prefaut
Laboratoire de Physiologie des Interactions
Hôpital Arnaud de Villeneuve et UPRES
EA 701, 371, Avenue du doyen G Giraud,
34295 Montpellier, France

Abstract *Objective:* We examined in rabbits the effects of more than 48 h of mechanical ventilation on the contractile properties and fiber type adaptations of the respiratory muscles. *Design and setting:* Experimental prospective study in a university laboratory. *Animals and interventions:* Nineteen rabbits were randomly allocated to two groups: control (n=10) or mechanically ventilated (MV; n=9) for 51±3 h. *Measurements and results:* Respiratory muscles contractile properties were analyzed before and after a fatigue protocol using in vivo isometric 1-s tetanic contraction characteristics in both muscles: peak tetanic force, contraction time, relaxation time, and total contraction time. Both muscle fiber type proportions, diameter, and cross-sectional areas were measured using ATPase staining. The MV rabbits showed significant weight loss in both muscles, accompanied by a reduced peak tetanic force (9.96±3.2 vs. 7.44±2.2 N for diaphragm of control and MV animals respectively), fatigue resistance index, and increased relaxation time (57.5±8.7 vs. 85.8±9.4 ms for diaphragm of control and MV animals) and contraction time. These impairments in the MV group worsened after the fatigue runs. Both muscle showed a significant atrophy of type IIa and IIb fibers but a stability in type I fibers cross-sectional area.
Conclusions: Mechanical ventilation in rabbits produces alterations in contractile properties of the diaphragm and 5th external intercostal muscle, increases both muscles fatigue, and promotes atrophy of type II fibers.

Keywords Diaphragm · Rib cage muscles · Muscle contraction · Respiratory muscles mass · Muscle fiber atrophy

Introduction

Mechanical ventilation (MV) is widely used for the treatment of respiratory failure. However, prolonged mechanical ventilation (PMV) seems to be one of the principal factors responsible for the difficult weanings [1, 2] encountered in as many as 25% of these patients [3, 4]. During controlled MV, respiratory muscle activity decreases dramatically, as demonstrated by the absence of diaphragmatic electrical activity [5, 6]. Diaphragmatic disuse during denervation and other forms of inactivation has been shown to affect contractile and morphometric properties of the muscle fibers [7, 8, 9]. Only limited information is available concerning the effects of disuse mediated by PMV on respiratory muscle mass, contractile properties, endurance capacity, and fibers type changes [10, 11, 12]. Le Bourdelles et al. [10] reported that 48 h of MV in rats was associated with decreased muscle weight and altered in vitro contractile properties of the diaphragm, while no effects were noted on the soleus and extensor digitorum longus muscles. Anzueto et al. [11] demonstrated that 11 days of MV resulted in decreased diaphragm muscle strength and endurance in baboons. The external intercostal muscles of

the upper rib cage, as in the parasternal intercostal muscles, are inspiratory agonists during eupneic breathing [13, 14]. During inspiratory loaded conditions, as seen during difficult weanings, the relative contribution of the rib cage muscles to the respiratory effort increases [15, 16], and predominant activation is possible. No data exist concerning the effect of PMV on rib cage muscle contractile properties.

The aims of this study were to determine whether more than 48 h of MV decreases the mass and contractile properties, increases fatigue, and changes fiber type of the diaphragm and 5th intercostal muscles in rabbits.

Methods

General procedures

We developed an original model of PMV in adults rabbits. Care of the animals conformed to the recommendations of the institutional animal care committee. Nineteen adult New Zealand rabbits (INRA, Montpellier, France) were studied. The animals were randomly divided into two groups: a group receiving MV (n=9) and a control group (n=10). The rabbits in the MV group were premedicated with intramuscular ketamine (50 mg/kg), diazepam (2 mg/kg), and atropine (0.125 mg) [17]. They were tracheotomized under general intravenous anesthesia (ketamine 7.5 mg/kg) and mechanically ventilated for more than 48 h with a volume-cycled ventilator (RP 200, LSA, Fontenay-sous-Bois, France) with 40% FIO_2, tidal volume at 8 ml/kg body weight, respiratory rate at 60 bpm, Ti/Ttot ratio at 1/1.5 and the addition of a positive end expiratory pressure at 2 cmH_2O [17]. Anesthesia was maintained during the MV period by a continuous intravenous infusion of sodium thiopental (15 mg/kg per hour). Neuromuscular blocking agents were not used. The authors established the parenteral nutrients according to veterinary research protocols adapted to experimental animals. Animals received 10 g proteins per day. The animals received continuous parenteral nutrition with a nutrient composition of 60% carbohydrate, 25% lipids, 15% proteins, and vitamins, minerals, and 50 mg/kg cefamandole. The daily administration was of 400 ml, equivalent to 400 kcal/day. Systolic blood pressure was continuously measured (E_1 130, Phillips, Amsterdam, The Netherlands) through a catheter placed in the ear central artery and maintained within a range of 90–120 mmHg. The animal's temperature was recorded continuously and maintained at 38°C with an electric blanket. The animals in the control group were free of intervention during the 48-h experimental period, with access to food and water ad libitum. They received during 1 h the same anesthetic protocol, the same instrumentation as the MV group, and were ventilated with the same ventilatory parameters before measurements and blood samples.

After 48 h the animals were weighed, and blood samples were taken from the artery for blood gas values and measurement of total protein, sodium, potassium, calcium, phosphorus, and magnesium. A laparotomy and an external left thoracic approach were then performed, and isometric contractile properties were measured. The costal and crural portions of the diaphragm and the 5th external intercostal muscle were then immediately removed, dissected, and weighed separately, and the animals were killed by exsanguination.

In vivo measurement of respiratory muscle contractile properties

The integrated electrical activity of the diaphragm was recorded continuously in five rabbits during the 48 h experimental period with two needle electrodes (Nicolet Instruments, Trappes, France) subcutaneously implanted on both sides of the lower part of the sternum. The electrodes were connected to a preamplifier (sample frequency 2048 Hz, band-pass filter: 10 Hz–1 KHz, attentuation >6 db/decade). Measurements on diaphragm and rib cage muscles were carried out in situ on muscles strips intact in their origin and insertions on the central tendon and/or on the ribs. Dessication of the preparation was avoided by saline humidification. For the diaphragm muscular strips (5 mm) from the right costal hemidiaphragm were selected and a nonextensible thread was set at the same location for each animal. The thread was connected to a strain gauges force transducer (INSERM U 103, Montpellier, France) located in the contraction axis of muscle fibers. The muscle strip was adjusted to the length at which maximal tetanic tension was elicited (preliminary unpublished studies). Resting (precontraction) muscle length and tension of both muscles could be altered by raising or lowering the force transducer. Direct stimulation was delivered via two implanted needle electrodes. The electrodes were connected to a current regulated electrical stimulator (STIPRO 10, St Mathieu de Treviers, France) which delivered biphasic charge balanced pulses (150 mA maximal output). Supramaximal (40 mA) square-wave pulses of 300 μs duration at 40 Hz frequency were delivered for 1 s (tetanic contraction). Five 1-s supramaximal tetanic contractions with a rest period of 5 s were recorded. After a 5-min rest the muscle was subjected to a fatigue run consisting of continuous stimulation at 40 Hz for 60 s. Another sequence of five 1-s tetanic contraction were then elicited 1 min after the fatigue run. An intercostal muscle strip (5 mm large) was adjusted to the length at witch maximal tetanic tension was elicited. For this strip the upper rib was fixed to an upright cork plate and the lower part of the muscle sutured to the strain gauge force transducer. The same protocol as for the diaphragm was used. Direct stimulation was delivered via two intramuscular needle electrodes distant from 0.5 cm.

Tetanic contractions of 1 s were chosen for study as, due to the contraction time (CT), relaxation time (RT), and total contraction time (TCT), they provide a more accurate index of changes occurring over the time course of contraction than the isometric twitch. The 1-s tetanic contractions allowed for the active state, rarely attained in mammalian muscle during twitch contractions, to be fully developed [18, 19]. Peak tetanic force (Po), CT (calculated from 10% to 90% of peak force), RT (calculated from 90% to 10% of peak force), and TCT were determined from the stored oscilloscope tracing of the1 s tetanic contraction (Tektronix 222, Beaverton, Ore., USA) and analyzed by a personal computer (PC Pentium 120 MHz). A fatigue resistance index (FRI) was calculated using fatigue force measured after a continuous 60-s electrical stimulation, divided by baseline force, measured prior to the fatigue run [18, 19]. Software written in Labview (National Instruments, Austin, Tex., USA) was used for the acquisitions. Signals coming from the force transducer were acquired through an input-output board for PC (12-bit resolution). Data were sampled at 1024 Hz (time acquisition window of 2 s). The signals were differentially amplified and low-pass filtered (1 kHz; Tektronix M 555).

Muscle fiber type proportions, diameters and cross-sectional areas

Once the physiological measurements were completed, muscle segments from the middle costal region of the diaphragm and 5th external intercostal muscle of each rabbit were pinned to a cork and quick-frozen in isopentane cooled by liquid nitrogen. Serial 10-μm-thick cross-sections were sliced from the middle of each muscle segment using a cryostat (HN500, Microm, Heldelberg, Germany) maintained at –20°C. The fields were cut perpendicularly to the myocites axis. Alternate sections were stained for myofibrillar adenosine triphosphatase (mATPase). Differences in mATPase staining after preincubation at pH 10.4 were used to classify the muscle fibers as either type I or II. Type II fibers were

subclassified as either type IIa or IIb using mATPase staining profiles following preincubation at pH 4.6. Fiber type proportions, minimal diameters, and cross-sectional areas (CSA) were then determined from a sample of 200–250 fibers per muscle. Fiber diameter was measured using a micrometric scale on 30 fibers of each type. The muscle sections were magnified ×20 (Carl Zeiss, Axioscop, Ulm, Germany) and then digitized using an image processing system (Panasonic, Tokyo, Japan, and Digital System, Institut de Biologie, Montpellier, France). The area of each pixel, measured by a stage micrometer, was 0.71 μm^2. Thirty fibers of each type were outlined, and fiber CSA was calculated from the number of pixels counted within each fibers outlined borders. The contribution of the interstitial space to the total muscle area was not considered.

Statistical analysis

Values were means ±SEM or means ±SD. Individual comparison between experimental and control rabbits were made using nonparametric tests (Mann-Whitney). Bonferroni's correction was used where necessary. Comparisons of individual muscle contractile properties before and after the fatigue runs were performed using the paired two-tailed Student's *t* test. The level of $p<0.05$ was accepted as statistically significant.

Results

MV rabbits were ventilated for 51±3 h. Throughout the study the rabbits of the MV group were afebrile, with body temperature ranging between 36°C and 38°C. They had an intestinal transit and urinated spontaneously. There was no difference between control and MV animals regarding plasmatic levels of total protein (57±4 vs. 51±9 g/l), sodium (148±3 vs. 147±5 mmol/l), potassium (4.7±0.4 vs. 4.1±0.3 mmol/l), calcium (2.7±0.2 vs. 3±0.2 mmol/l), magnesium (1.4±0.3 vs. 1.7±0.2 mmol/l), or phosphorus (2±0.2 vs. 1.8±0.1 mmol/l). There were also no statistical significant differences in blood gases (PaO_2, 139±25 vs. 167±33 mmHg; $PaCO_2$, 38±4 vs. 37±7 mmHg; pH, 7.35±0.1 vs. 7.37±0.1) and hemodynamic data (heart rate, 179±13 vs. 188±14 bpm; systolic blood pressure, 103±17 vs. 108±21 mmHg).

Muscle and body weight

The initial body weights of the control and MV rabbits showed no significant difference (respectively, 2.630±212 and 2.710±170 g). Following the experimental period the body weights of both group decreased, showing no significant intergroup differences (respectively, 2.510±170 and 2.570±190 g). However, the weights of the two portions of the diaphragm and the 5th external intercostal muscle were significantly reduced in the MV group ($p<0.05$; Table 1).

Table 1 Muscle mass in mechanically ventilated (MV) and in control animals (means ±SD)

	MV (n=9)	Control (n=10)	p
Costal diaphragm			
Absolute value (g)	2.9±0.3	3.7±0.4	<0.01
Mass/body weight (×10^3)	1.12±0.2	1.57±0.2	<0.05
Crural diaphragm			
Absolute value (g)	1.2±0.3	1.6±0.3	<0.05
Mass/body weight (×10^3)	0.49±0.1	0.65±0.1	<0.05
5th left external intercostal			
Absolute value (g)	0.5±0.1	0.7±0.1	<0.05
Mass/body weight (×10^3)	0.19±0.03	0.27±0.07	<0.05

Contractile properties

No electrical activity during MV was noted in the diaphragm. The electromyographic (EMG) tracing were recorded before and during MV. There was an absence of phasic activity during MV. The contractiles properties of the diaphragm and rib cage muscles are shown in Table 2.

Intergroup comparison

MV significantly decreased Po compared to controls (25%) and increased CT, RT, and TCT of the diaphragm before the fatigue run (Table 2). After the fatigue run the Po of MV animals decreased by 47% and CT, RT, and TCT significantly increased compared with the control group. Prior to the fatigue runs the MV rabbits showed significant decreases in Po (10%) and significant increases in RT and TCT of the 5th external intercostal muscle (Table 2) compared with the control group. After the fatigue run the difference between MV and control animals for Po was 19%. The FRI values were found to be significantly higher in the control rabbits

Intragroup comparison

The control rabbits' tetanic force 1 min after the fatigue run was decreased by 27% ($p<0.05$) of the baseline value, while CT was increased by 40% ($p<0.05$). The MV rabbits showed a significantly different decrease in Po (43%, $p<0.05$) and increase in RT (45%, $p<0.05$); CT increased by 49% (n.s. vs. controls). The 1-s tetanic response tests of control rabbit 1 and MV rabbit 3 are reported in Fig. 1a.

In control rabbits Po generated by the 5th external intercostal muscles 1 min after the fatigue run was decreased by 29% of the baseline value ($p<0.05$) while RT was increased by 45% ($p<0.05$). MV rabbits' Po was de-

Table 2 Contractile properties in mechanically ventilated (MV) and control (control) animals before and after fatigue runs (*Po* peak tetanic force, *CT* contraction time, *RT* relaxation time, *TCT* total contraction time, *FRI* fatigue resistance index) (means ±SEM)

	Diaphragm		Intercostal muscle	
	MV	Control	MV	Control
Before fatigue				
Po (N)	7.44±2.2*	9.96±3.2	12.5±3.47*	13.9±3.9
Po/g muscle strip (N/g)	44.5±13.3*	51.6±21	215.5±43	195±37
CT (ms)	244±54*	184±55.5	273±54.1	267.2±82.4
RT (ms)	85.8±9.4*	57.5±8.7	87.8±19.6*	77.1±26.9
TCT (ms)	930.7±83*	839.1±93	963.1±16.7*	931.7±52.1
After fatigue				
Po (N)	4.3±1.5*,**	8.18±1.5**	7.53±2.98*,**	9.27±3.47**
Po/g muscle strip (N/g)	25.7±10*,**	42.4±17**	129±27**	127±19**
CT (ms)	365.6±77.7*,**	296.4±91**	379.1±76*,**	257.7±72.4
RT (ms)	124.6±19.4*,**	64.7±18.3	168.6±25.6*,**	112.5±25.6**
TCT (ms)	961.2±45.6*	888.9±58.4	1113±136*,**	831.6±151.1
FRI (%)	54±7*	79±9	58±5*	64±5

* $p<0.05$ MV vs. control,
** $p<0.05$ before vs. after fatigue

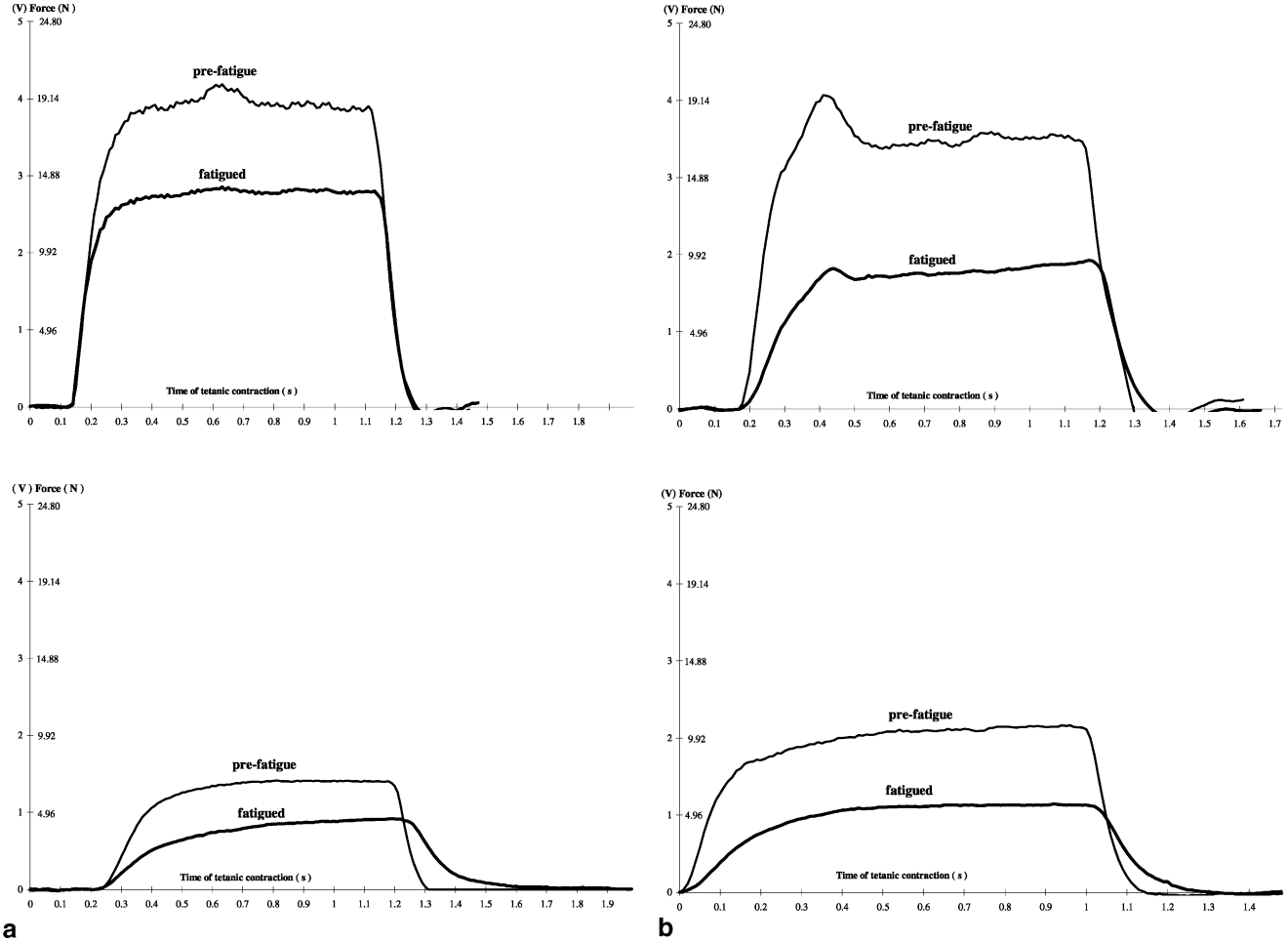

Fig. 1 Diaphragmatic (**a**) and intercostal muscle (**b**) 1-s tetanic contraction at 40 Hz before and 1 min after the fatigue protocol in a control animal (rabbit no. 1, *top panel*) and a mechanically ventilated (MV) animal (rabbit no. 3, *bottom panel*). Note the prefatigue decrease in peak tetanic force in this MV rabbit acompanied by increased contraction and relaxation times compared to the control animal. While all contractiles properties were compromised after the fatigue run in the MV rabbit, only the peak force decreased in the control rabbit

Table 3 Effects of mechanical ventilation on fiber type proportions, minimal diameters, and cross-sectional area (*CSA*) in diaphragm and 5th external intercostal muscle (means ±SD)

	Diaphragm		Intercostal muscle	
	MV	Control	MV	Control
Type proportion (%)				
Type 1	49.±2.1	48.4±2.7	37.1±2.4	38.3±2
Type IIa	20.2±3.1	21.4±1.7	30.9±1.7	30.7±1.9
Type IIb	30.5±2.7	30.2±1.9	32±2.1	31±2.4
Minimal diameter (μm)				
Type I	41±3	44±3	39±4	43±3
Type IIa	51±1*	57±3	49±5*	53±3
Type IIb	50±2*	58±2	54±2	57±4
CSA (μm^2)				
Type I	1356±81	1524±107	1287±211	1479±112
Type IIa	2041±117*	2540±317	1956±184*	2372±301
Type IIb	2097±119*	2620±219	2412±301*	2682±257

*$p<0.05$ MV vs. control

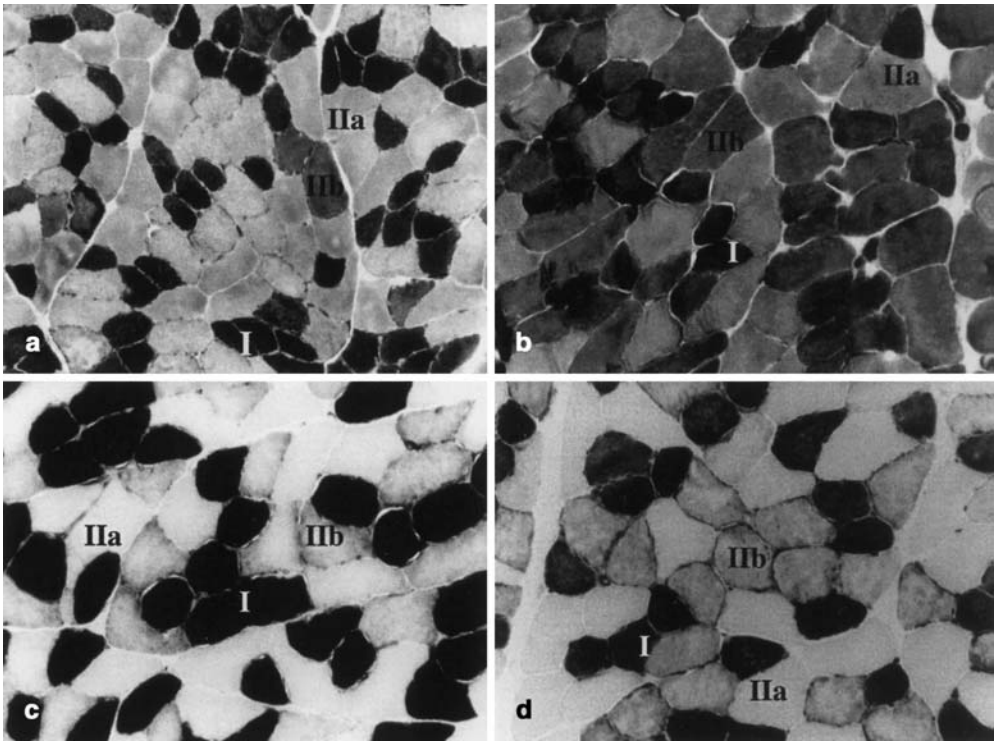

Fig. 2a–d Histochemical sections (×20) stained for myosin ATPase after preincubation at pH 4.6. *Dark staining* Type I fibers appear dark; *light staining* type IIa fibers; *intermediate staining* type IIb fibers. **a** Diaphragm of a MV rabbit. **b** Diaphragm of a control rabbit. **c** Intercostal muscle of a MV rabbit. **d** Intercostal muscle of a control rabbit. Note that type II fibers appear mainly smaller in costal diaphragm and in a lesser manner in intercostal muscle. The sections shown above were chosen to illustrate the preferential atrophy of type II fibers without modification of the selective composition of the fiber types

creased by 41% ($p<0.05$) of baseline while CT and RT were increased by 38% and 93% respectively ($p<0.05$; Table 2). The 1-s tetanic response tests of control rabbit 1 and MV rabbit 3 are reported in Fig. 1a for the diaphragm and Fig. 1b for the 5th intercostal muscle.

Muscle fiber type proportions, diameter, and CSA

Data concerning muscle fibers type proportions, minimal diameter, and CSA are reported in Table 3. Differences were noted in the fiber type proportions of the two muscles. Type I and IIa fibers accounted, respectively, for 50% and 20% of all diaphragm fibers and for 40% and 30% of 5th external intercostal muscle fibers. Type IIb fibers accounted for 30% of all fibers in both muscles.

After PMV the fiber type proportions of neither muscle was significantly altered. Figure 2 presents photomicrographs of typical diaphragm and rib cage muscle cross-sections following preincubation at pH 4.6. For both muscles the differences in diameter and CSA were noted across fiber types in both MV and control rabbits. Type I fibers were the smallest followed in rank order by the IIa and IIb fibers ($p<0.05$). No significant differences in type I fiber diameter or CSA were noted between the groups in either muscle. The decrease in CSA of the type I fibers in the MV group was 8±1.5% and 9±1% for the diaphragm and rib cage muscle, respectively. In contrast, the MV rabbits showed a significant decrease in the CSA of type IIa and IIb fibers of the diaphragm and intercostal muscles: respectively, IIa fibers 21±3% and 16±2%; IIb fibers 23±4% and 11±2% ($p<0.05$). The decrease in CSA of type II fibers was greater in the diaphragm than in the rib cage muscles.

Discussion

The principal findings of this study are that 51±3 h of MV in an original model of PMV rabbits leads itself to significant changes in the diaphragm and 5th external intercostal muscles: reduced weights, reduced force generating capacities and fatigue resistance, and atrophy of type IIa and IIb fibers. In accordance with similar findings reported in the literature [10, 11], MV appears to alter diaphragmatic and rib cage muscle function in rabbits. Using in vitro twitch and force frequency curves, Le Bourdelles et al. [10] showed that 48 h of MV was associated with significant decreases in diaphragm force but without alterations in half-RT or modifications in muscle protein concentrations and enzymatic activities. These findings were not observed in the extensor digitorum longus (fast twitch) and soleus (slow twitch) muscles. However, comparing in vivo and in vitro contractile properties is difficult, involving alterations in anatomical shape and thermolability of the contractile properties [20]. Anzueto et al. [11] found a decrease in transdiaphragmatic pressure and in diaphragm muscle endurance after 12 days of MV in three baboons. However, as the authors measured diaphragm contractility by indirect bilateral phrenic nerve rather than direct muscle stimulation, it has been suggested that the neural command was altered [18], and end-plate failure was present in the disused muscle [9]. Furthermore, their use of long lasting neuromuscular blockers may have contributed to the decreased diaphragmatic force.

The present study found that MV in an in vivo rabbit model was associated with a significant reduction in Po and slowing of CT, RT, and TCT. These alterations, as demonstrated in other diaphragmatic denervation models [7, 8, 9], significantly worsened after a fatigue run. While confirming the reduced diaphragmatic force following MV found in other animal models [10, 11], our results demonstrate alterations in rib cage muscle force, where contractile properties are of particular clinical interest. Rib cage muscles act as force generators during resistive loaded ventilation [15, 16]. During difficult weanings following PMV [2, 21] a preferential activation of the rib cage muscles has been demonstrated. A period of PMV should promote alterations in the contractile properties of rib cage muscles in such situations.

Respiratory muscle inactivity during MV should alter contractile properties. As observed in our MV group, respiratory muscle activity has been shown totally to cease during controlled MV, as revealed by the absence of respiratory muscle EMG signals during respiratory support [5, 6]. While MV should reverse respiratory muscle fatigue by allowing them to rest, it may also result in their impaired contractility. Marked alterations in diaphragmatic contractile properties have been observed in several models of diaphragm muscle inactivation [7, 8, 9, 22]. Most notably, decreases in specific force and increases in twitch contraction and half-RT were demonstrated. Muscular inactivity in our MV group may have had an effect on the excitation-contraction coupling of both diaphragm and rib cage muscles, but we have no data to support this hypothesis. Diaphragm contractility is also thought to depend on extracellular Ca^{2+} concentration [23]. However, no effect on rib cage muscles was observed, and no significant differences were noted in our study's plasma Ca^{2+} levels between the control and MV animals.

Respiratory muscle contractile impairment could be related to starvation leading to weight loss and hydration or metabolic alterations. Decreases in diaphragm weight mediated by acute nutritional deprivation remain uncertain [24, 25]. When compared with control animals, diaphragms weighed 25% more following 2 weeks of denervation, but the diaphragm weight decreased with the association denervation-malnutrition [26]. In accordance with the findings of Le Bourdelles et al. [10], we found a significant decrease in the muscle mass of the two parts of the diaphragm tested and 5th external intercostal muscle in the MV group. No changes in diaphragm water content have been reported following denervation or muscular inactivity [27] that could explain changes in diaphragm weight. In our animals the force reduction for the diaphragm appears to be almost completely related to change in mass. For the intercostal muscle the MV group had 5–10% more force/g muscle strip than did the control group. We have no clear explanation for the difference between the muscles concerning force generation per gram. Diaphragmatic impairment related to weight and intercostal muscle impairment not related to weight in MV animals could be related to the prior history of activation of both muscles. Optimal and total length of these muscles may differ between control and MV groups [10].

During a 40 Hz electrical stimulation both slow (type I) and fast (type II) twitch motor units are recruited [9]. The respiratory muscles fatigue resistance index noted in our MV rabbits decreased despite the reduction of the type II fiber CSA, without changes in either the type I fiber CSA or the fiber type ratio, yielded a larger proportional area of type I fibers. These results are contradictory with those of 2-week denervation models [7, 8, 20, 28]. Two weeks of hemidiaphragm inactivity, induced by either tetrodotoxin or phrenic transection, left the proportions of type I and II fibers unaffected while the type I fibers hypertrophied, the type II fibers atrophied, and the fatigue resistance increased. Three days of denervation in a rat model is reported to be associated with an initial hypertrophy of all fiber types [22, 25, 29], without changes in fiber type proportions. This difference with the present study, involving an acute period of disuse, may be explained by the employed diaphragm muscle inactivity model. In hemidiaphragm denervation models the fiber type changes may result from the passive stretching and consequent mechanical loading imposed on the muscle fibers by the increased inspiratory motion of the intact contralateral side of the diaphragm [7]. In denervation models communication between motoneuron and muscle is completely abolished, and when tetrodotoxin is used, the phrenic nerve increases its activity by approximately 60% [9]. In contrast, when using PMV, the communication between motoneuron and muscles persists while both are inactivated. These differences are of importance as most theories concerning fiber type disuse adaptations involve actions of transported neurotrophic substances and synthesized myotrophic factors [28]. It has been demonstrated that the diaphragm's type I fibers hypertrophied while the type II fibers atrophied in tetrodotoxin and denervation models [30]. In humans these results would differ, but diaphragm and rib cage muscles have similar fiber type compositions [31]. Furthermore, it has been demonstrated that the percentage of type I fibers decreases while that of type II fibers increases in the rostrocaudal direction from the 1st through 6th intercostal spaces [32]. As such, the result in fiber type proportions depends of which intercostal muscle is studied.

A relationship has been demonstrated between myosin heavy chain phenotypes and the contractile properties of muscle fibers [9]. Modifications in the myosin isoform composition have been associated with 3–7 days of MV in rats [33]. Hybrid fibers expressing both type I and II myosin heavy chains increased (12.5% vs. 3% in control) at the expense of the type II fiber population. These alterations may modify the maximal specific force and fatigue resistance seen in our MV group.

Finally, respiratory muscle contractile functions could be altered by the use of pentobarbital. In vitro studies on skeletal muscles demonstrated that the drug potentiated twitch amplitude, increased the rate of twitch tension development, and reduced the rate of twitch relaxation [34]. Pentobarbital was also shown to affect external intercostal activation without altering peak diaphragm EMGs in dogs [35]. In the present study all rabbits received thiopental anesthesia, and both muscles showed reduced tetanic strength, and increased RT. The data suggest that thiopental was not involved in the genesis of respiratory muscle contractile alterations.

In summary, 51 h of MV in rabbits were associated with changes in the contractile properties and fiber type of the respiratory muscles. These adaptations may affect the ability to wean some patients from MV. Further studies are needed to determine the role of PMV on respiratory muscle performance.

Acknowledgements This research was presented in part at the Annual Meeting of the American Thoracic Society, San Francisco, California, May 1998, and at the 40th National Congress of the French Society of Anesthesiology and Intensive Care Medicine, Paris, France, 22–25 September 1998

References

1. Capdevila X, Perrigault PF, Peray PJ, Roustan JP, d'Athis F (1995) Occlusion pressure and its ratio to maximum inspiratory pressure are useful predictors for successful extubation following T-piece weaning trial. Chest 108:482–489
2. Capdevila X, Perrigault PF, Ramonatxo M, Roustan JP, Peray P, d'Athis F, Prefaut C (1998) Changes in breathing pattern and respiratory muscle performance parameters during difficult weaning. Crit Care Med 26:79–87
3. Brochard L, Rauss A, Benito S, Conti G, Mancebo J, Rekik N, Gasparetto A, Lemaire F (1994) Comparison of three methods of gradual withdrawal from ventilatory support during weaning from mechanical ventilation. Am J Respir Crit Care Med 150:896–903
4. Esteban A, Frutos F, Tobin M, Alia I, Solsona JF, Valverdu I, Fernandez R, de la Cal M, Benito S, et al (1995) A comparison of four methods of weaning patients from mechanical ventilation. N Engl J Med 332:345–350
5. Brochard L, Harf A, Lorino H, Lemaire F (1989) Inspiratory pressure support prevents diaphragmatic fatigue during weaning from mechanical ventilation. Am Rev Respir Dis 139:513–521
6. Flick GR, Bellamy PE, Simmons DH (1989) Diaphragmatic contraction during assisted mechanical ventilation. Chest 96:130–135

7. Zhan WZ, Farkas GA, Schroeder MA, Gosselin LE, Sieck GC (1995) Regional adaptations of rabbit diaphragm muscle fibers to unilateral denervation. J Appl Physiol 79:941–950
8. Zhan WZ, Sieck GC (1992) Adaptations of diaphragm and medial gastrocnemius muscles to inactivity. J Appl Physiol 72:1445–1453
9. Sieck GC (1994) Physiological effects of diaphragm muscle denervation and disuse. Clin Chest Med 15:641–659
10. Le Bourdelles G, Viires N, Boczkowski J, Seta N, Pavlovic D, Aubier M (1994) Effects of mechanical ventilation on diaphragmatic contractile properties in rats. Am J Respir Crit Care Med 149:1529–1544
11. Anzueto A, Peters JI, Tobin MJ, De Los Santos R, Seidenfeld JJ, Moore G, Cox WJ, Coalson JJ (1997) Effects of prolonged controlled mechanical ventilation on diaphragmatic function in healthy adult baboons. Crit Care Med 25:1187–1190
12. Ferrigno M, Bishop B, Morin FC (1994) Diaphragmatic function during prolonged mechanical ventilation in non-paralyzed ewes (abstract). Anesthesiology 81:A322
13. Di Marco AF, Romaniuk JR, Supinski GS (1990) Parasternal and external intercostal muscle shortening during eupneic breathing. J Appl Physiol 69:2222–2226
14. De Troyer AF, Farkas GA (1989) Inspiratory function of the levator costae and external intercostal muscles in the dog. J Appl Physiol 67:2614–2621
15. Hershenson MB, Kikuchi Y, Tzelepis GE, McCool FD (1989) Preferential fatigue of the rib cage muscles during inspiratory resistive loaded ventilation. J Appl Physiol 66:750–754
16. Zocchi L, Fitting JW, Majani U, Fracchia C, Rampulla C, Grassino A (1993) Effect of pressure and timing of contraction on human rib cage muscle fatigue. Am Rev Respir Dis 147:857–864
17. Flecknell PA (1993) Anaesthesia of animals for biomedical research. Br J Anaesth 71:885–894
18. Duchateau J, Hainaut K1987 Electrical and mechanical changes in immobilized human muscle. J Appl Physiol 62:2168–2173
19. Robinson GA, Enoka RM, Stuart DG (1991) Immobilization-induced changes in motor unit force and fatigability in the cat. Muscle Nerve 14:563–573
20. Prezant DJ, Richner B, Valentine DE, Aldrich TK, Fischman CL, Nagasthima H, Chaudhry I, Cahill J (1990) Temperature dependence of rat diaphragm muscle contractility and fatigue. J Appl Physiol 69:1740–1745
21. Tobin MJ, Perez W, Guenther SM, et al (1986) The pattern of breathing during successful and unsuccessful trials of weaning from mechanical ventilation. Am Rev Respir Dis 134:1111–1118
22. Gosselin LE, Brice G, Carlson B, Prakash YS, Sieck GC (1994) Changes in satellite cell mitotic activity during acute period of unilateral diaphragm denervation. J Appl Physiol 77:1128–1134
23. Viires N, Murciano D, Seta JP, Dureuil B, Pariente R, Aubier M (1988) Effects of Ca2+ withdrawal on diaphragmatic fiber tension generation. J Appl Physiol 64:15–19
24. Prezant DJ, Valentine DE, Kim HH, Gentry EI (1993) Effects of starvation and refeeding on adult male rat diaphragm contractility, fatigue, and fiber types. J Appl Physiol 74:742–749
25. Lewis MI, Sieck GC (1990) Effect of acute nutritional deprivation on diaphragm structure and function. J Appl Physiol 68:1938–1944
26. Lewis MI, Lorusso TJ, Zhan WZ, Sieck GC (1996) Interactive effects of denervation and malnutrition on diaphragm structure and function. J Appl Physiol 81:2165–2172
27. Sola OM, Martin AW (1953) Denervation hypertrophy and atrophy of the hemidiaphragm of the rat. Am J Physiol 172:324–332
28. Miyata H, Zhan WZ, Prakash YS, Sieck GC (1995) Myoneural interactions affect diaphragm muscle adaptations to inactivity. J Appl Physiol 79:1640–1649
29. Yellin H (1974) Changes in fiber types of the hypertrophying denervated hemidiaphragm. Exp Neurol 42:412–428
30. Ibebunjo C, Martyn JAJ (1999) Fiber atrophy, but not changes in acetylcholine receptor expression, contributes to the muscle dysfunction after immobilization. Crit Care Med 27:275–285
31. Sanchez J, Derenne JP, Debese B, Riquet M, Monod H (1982) Txpology of the respiratory muscles in normal men and in patients with moderate chronic respiratory diseases. Bull Eur Physiopathol Respir 18:901–914
32. Kelsen SG, Bao S, Thomas AJ, Mandini IA, Griner GJ (1993) Structure of parasternal intercostal muscles in the adult hamster: topographic effects. J Appl Physiol 75:1150–1154
33. Yang L, Luo J, Lin MC, Gottfried SB, Petrof BJ (1997) Effect of long-term mechanical ventilation on rat diaphragm mass and myosins heavy chain expression (abstract). Am Rev Respir Crit Care Med A509
34. Taylor RG, Abresch RT, Lieberman JS, Fowler WMJ, Portwool MM (1984) Effects of pentobatbital on contractility of mouse skeletal muscle. Exp Neurol 83:254–263
35. Di Marco AF, Supinski GS, Kowalski KE, Romaniuk JR (1994) Effects of pentobarbital anesthesia on intercostal muscle activation and shortening. J Appl Physiol 77:925–932