ORIGINAL

Effects of levosimendan on respiratory muscle function in patients weaning from mechanical ventilation

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Abstract

Purpose: Respiratory muscle weakness frequently develops in critically ill patients and is associated with adverse outcome, including difficult weaning from mechanical ventilation. Today, no drug is approved to improve respiratory muscle function in these patients. Previously, we have shown that the calcium sensitizer levosimendan improves calcium sensitivity of human diaphragm muscle fibers in vitro and contractile efficiency of the diaphragm in healthy subjects. The main purpose of this study is to investigate the effects of levosimendan on diaphragm contractile efficiency in mechanically ventilated patients.

Methods: In a double-blind randomized placebo-controlled trial, mechanically ventilated patients performed two 30-min continuous positive airway pressure (CPAP) trials with 5-h interval. After the first CPAP trial, study medication (levosimendan 0.2 µg/kg/min continuous infusion or placebo) was administered. During the CPAP trials, electrical activity of the diaphragm (EA_{di}), transdiaphragmatic pressure (P_{di}), and flow were measured. Neuromechanical efficiency (primary outcome parameter) was calculated.

Results: Thirty-nine patients were included in the study. Neuromechanical efficiency was not different during the CPAP trial after levosimendan administration compared to the CPAP trial before study medication. Tidal volume and minute ventilation were higher after levosimendan administration (11 and 21%, respectively), whereas EA_{di} and P_{di} were higher in both groups in the CPAP trial after study medication compared to the CPAP trial before study medication.

Conclusions: Levosimendan does not improve diaphragm contractile efficiency.

Keywords: Diaphragm, Critical-illness-associated respiratory muscle weakness, Mechanical ventilation weaning, Calcium sensitization, Levosimendan

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Introduction

Respiratory muscle weakness frequently develops in ventilated critically ill patients and is associated with adverse outcome, including difficult weaning from mechanical ventilation [1–10]. Therefore, strategies that aim to improve respiratory muscle function are much needed. Today, no drug is approved for the treatment of respiratory muscle weakness [11].

Muscle weakness may result from loss of contractile proteins (atrophy) or contractile protein dysfunction. Indeed, atrophy of the diaphragm has been demonstrated in critically ill ventilated patients using ultrasound [8] and muscle biopsies [12, 13]. Recently, we found evidence for contractile protein dysfunction in diaphragm muscle fibers of ventilated intensive care unit (ICU) patients [12]. Diaphragmatic muscle fibers of these patients exhibit reduced calcium sensitivity of contraction [14], resulting in decreased force generation at submaximal intracellular calcium concentration [12]. Levosimendan, a cardiac inotrope that augments the calcium sensitivity of the troponin complex, has been shown to improve cardiac muscle contractility and is approved for clinical application worldwide for treatment of heart failure [15, 16].

In an experimental study, we demonstrated that levosimendan improves in vitro force generation of slow and fast twitch muscle fibers obtained from the diaphragm of patients with chronic obstructive pulmonary disease (COPD), but also from fibers obtained from elective surgery patients without comorbidities [17]. More recently, we demonstrated that levosimendan improves in vivo contractile efficiency of the diaphragm in healthy subjects [18]. Whether levosimendan improves in vivo contractile efficiency of the diaphragm in ICU patients has not been evaluated. Based on our previous studies in vitro [17] and in vivo [18], we hypothesized that levosimendan improves diaphragm contractile efficiency in patients weaning from mechanical ventilation. We conducted a double-blinded randomized placebo-controlled trial that aims to investigate the effects of levosimendan on respiratory muscle function, especially diaphragm contractile efficiency in ventilated ICU patients.

Methods
Study design and population
The study was a double-blind randomized placebo-controlled trial conducted in the ICU of the Radboud University Nijmegen Medical Center. Inclusion criteria were invasive mechanical ventilation for at least 3 days, inspiratory pressure support $(PS) \leq 10$ cmH$_2$O, positive end-expiratory pressure $(PEEP) \leq 10$ cmH$_2$O, P/F ratio ≥ 200 mmHg, hemodynamically stable (systolic blood pressure ≥ 110 mmHg without or with low dose

Take-home message

The calcium sensitizer levosimendan is approved as a cardiac inotrope, but preliminary studies have shown that it may also improve respiratory muscle function. This randomized placebo controlled trial shows that levosimendan improves tidal volume, minute ventilation and lowers arterial CO$_2$ in patients weaning from mechanical ventilation.

vasopressors), and able to sustain a 30-min continuous positive airway pressure (CPAP) trial. Exclusion criteria were neuromuscular disorder before ICU admission, new onset cardiac arrhythmias, left ventricle ejection fraction $<35\%$, severe kidney failure (serum creatinine >150 μmol/L), pregnancy or contra-indication for the placement of nasogastric tube (upper airway or esophageal pathology, recent nasal bleeding). The protocol was approved by the local ethical committee and registered at ClinicalTrials.gov (NCT01721434). Written informed consent was obtained from the legal representative before inclusion.

Trial design
Patients were ventilated with the Servo-i ventilator (Maquet, Sölna, Sweden). After enrollment, a multi-electrode nasogastric catheter with esophageal and gastric balloon (NeuroVent Research Inc, Toronto, Canada) was inserted through the nose. The catheter was positioned to obtain the best electrical activity of the diaphragm (EA$_{di}$) signal using software supplied with the ventilator [19]. EA$_{di}$ is a processed electromyographic (EMG) signal obtained from an esophageal multi-electrode recording of the diaphragm. The exact processing algorithm is proprietary, but roughly based on the processing algorithms of Sinderby and colleagues [20–22]. All patients were already equipped with an arterial catheter for blood pressure monitoring, a pulse oximeter, and ECG monitoring. Allocation to placebo or levosimendan occurred by block design (ratio of 1:1 to receive placebo or levosimendan). Placebo (riboflavin sodium phosphate 0.4 mg, dehydrated alcohol 100 mg and water 1 mL), and levosimendan were indistinguishable (both yellowish) to assure appropriate blinding. Each patient performed two 30-min CPAP trails with 5 h in between. During these two CPAP trials, inspiratory assist was reduced to zero, while PEEP level was maintained as determined by the treating physician. Thirty minutes after the first CPAP trial [i.e., at study time (hours:minutes) $T=1:00$], study medication [levosimendan 0.2 μg/kg/min or placebo (equal volume)] was administered intravenously till 30 min after the second CPAP trial. Arterial blood was withdrawn before and after CPAP trials. After the second CPAP trial, the study ended and standard clinical treatment was continued.

Data acquisition

Airway flow and processed EAdi were obtained (sampling rate 100 Hz) by connecting a RS-232 cable via the serial port of the Servo-i to a personal computer and resampled to 2 kHz. Esophageal (P_{es}) and gastric pressures (P_{ga}) were obtained (sampling rate 2 kHz) from the two balloons mounted on the esophageal catheter and connected to two differential pressure transducers (range ± 50 kPa, Freescale, Tempe, AR) and A/D converter (DT3004; Data Translation, USA). All signals were acquired continuously and synchronously using dedicated software (NeuroVent Research Inc, Toronto, Canada). Signals were analyzed offline using Matlab R2014b (Mathworks, Natick, MA, USA).

Data analysis

Transdiaphragmatic pressure (P_{di}) was calculated as P_{ga} minus P_{es}, from this, ΔP_{di} was defined as the difference between the start of increase in P_{di} and the peak value of P_{di} during inspiration. Inspiratory flow was integrated for the calculation of tidal volume (TV). Respiratory rate, minute ventilation, and inspiratory time (T_i) were determined from the flow signal. ΔEA_{di} was computed as the peak from the processed EA_{di} signal obtained from the Servo-i during inspiration. Neuromechanical efficiency (NME) was calculated as $\Delta P_{di}/\Delta EA_{di}$. Intrinsic PEEP (PEEPi) was computed as the difference in pressure between start of increase in P_{di} and start of inspiratory flow [23]. $P_{0.1}$, a measure of respiratory drive, was calculated as the decrease in P_{es} during the first 100 ms of inspiration [24]. Dynamic lung compliance (C_{dyn}) was computed as $TV/\Delta P_{es}$.

Data were analyzed on a breath-by-breath basis and averaged over a 2-min period free of artifacts or esophageal or gastric contractions. For each CPAP trial, four points in time were analyzed: study time (hours:minutes) $T = 0:01$, $0:05$, $0:15$, and $0:30$ for CPAP 1 and $T = 5:31$, $5:35$, $5:45$, and $6:00$ for CPAP 2.

Sample size calculation and statistical analysis

The primary outcome parameter is the difference in diaphragm NME response between placebo and levosimendan. NME was calculated at the end of both 30-min CPAP trials before and after study medication. Previously, we found a mean NME of 1.3 cmH$_2$O/μV with a standard deviation of 0.5 cmH$_2$O/μV, in response to administration of levosimendan neuromechanical efficiency increased by 21% [18]. Therefore, if we expect in the current study an increase in neuromechanical efficiency of 25%, this would mean a minimal detected difference of 0.33 cmH$_2$O/μV. When we assume a type I error of 0.05 (two sided) and type II error of 0.80, the calculated sample size is 19 patients per study arm.

Statistical analysis was performed using IBM SPSS Statistics version 22 (IBM Corp, Armonk, New York, USA). Continuous variables were compared using a Student's t test in case data had a normal distribution and Mann–Whitney U test in case of a non-normal distribution. A Chi-square test with either normal approximation or Fisher's exact test was used to compare nominal categorical variables. To assess the effect of levosimendan on respiratory and hemodynamic parameters, a two-way repeated measures ANOVA was used, with time (for respiratory parameters study time (hours:minutes) $T = 0:30$ and $6:00$; in addition, for hemodynamic parameters also at $T = 0:01$, $1:00$ and $5:31$) as the within-subject factor and group (study medication) as the between-subject factor. In case data were non-normally distributed, first, a log transformation was performed to obtain a normal distribution. Data are presented as mean $\pm$ SEM in case of a normal distribution and median (IQR) as stated otherwise. A two-tailed $P < 0.05$ was considered statistically significant.

Results

Patient characteristics

Thirty-nine subjects received study medication and completed the entire study protocol. Baseline characteristics for both groups are shown in Table 1. Two subjects were excluded from analysis of the primary endpoint (one from the placebo and one from the levosimendan group), one due to technical issues related to the nasogastric catheter, and another due to low EA_{di} precluding analysis of the primary endpoint. From the remaining subjects, 18 received placebo and 19 received levosimendan.

Effects of levosimendan on respiratory variables

Table 2 shows the absolute NME ($\Delta P_{di}/\Delta EA_{di}$) and Fig. 1a shows the normalized NME (NME normalized with respect to start of the CPAP trial without study medication [i.e. $T = 0:01$] over the course of the study. As expected, NME before study medication was not different between groups at start of the first CPAP trial and remained stable during the first CPAP trial in both groups. Levosimendan did not significantly affect diaphragm NME ($P = 0.407$) (from 1.2 ± 0.2 to 1.1 ± 0.2 cmH$_2$O/μV) compared to placebo (from 1.7 ± 0.5 to 2.1 ± 0.8 cmH$_2$O/μV) assessed after 30 min of CPAP.

ΔP_{di} and ΔEA_{di} were higher in both the levosimendan and placebo groups in the CPAP trial after study medication as compared to before study medication (Fig. 1b and Table 2).

Table 1 Patient characteristics

	Placebo ($n=19$)	Levosimendan ($n=20$)	P value
Age (yr)	63 (51–71)	67 (56–69)	0.53
BMI (kg/m^2)	26.0 (23.6–28.9)	24.6 (23.3–27.3)	0.69
Gender (M/F)	13/6	10/10	0.24
Reason ICU admittance, no. (%)			
Neurological	3 (16)	0 (0)	0.11
Pneumonia	1 (5)	4 (20)	0.34
Sepsis non-respiratory focus	3 (16)	3 (15)	>0.99
Post-surgery	4 (21)	6 (30)	0.52
Exacerbation COPD	2 (11)	0 (0)	0.23
Trauma	4 (21)	4 (20)	>0.99
Other	2 (11)	3 (15)	>0.99
Days of MV on study day	11 (7–23)	12 (5–18)	0.61
PS level (cmH$_2$O)	6 (6–10)	8 (6–9.5)	0.75
PEEP level (cmH$_2$O)	6 (5–10)	7 (5–8)	0.55
PaO$_2$/FiO$_2$ ratio (mmHg)	268 (233–306)	319 (256–377)	0.08
Comorbidities, no. (%)			
COPD	8 (42)	4 (20)	0.14
Diabetes mellitus	4 (21)	4 (20)	>0.99
Cancer	1 (5)	2 (11)	>0.99
Relevant treatment at time of study, no. (%)			
Norepinephrine	2 (11)	6 (30)	0.24
Steroids	6 (32)	3 (15)	0.27
Dobutamine	0 (0)	0 (0)	>0.99
Milrinon	0 (0)	0 (0)	>0.99
RASS CPAP trial before study medication	0 (−1 to 0)	−1 (−3 to 0)	0.04
RASS CPAP trial after study medication	0 (−1 to 0)	−1 (−3 to 0)	0.09
Delirious, no. (%)	1 (5)	5 (25)	0.18

Data are presented as median (IQR)

BMI body mass index, *MV* mechanical ventilation, *PS* pressure support, *PEEP* positive end-expiratory pressure, *COPD* chronic obstructive pulmonary disease, *RASS* Richmond Agitation Sedation scale

Before study medication, tidal volume was equal between groups during the CPAP trial, but after study medication, there was a larger increase ($P=0.017$) in tidal volume during CPAP in the levosimendan group (from 392 ± 22 to 437 ± 23 mL) compared to the placebo group (from 404 ± 32 to 418 ± 33 mL) (Fig. 1c). In addition, minute ventilation increased significantly more after levosimendan administration ($P=0.021$) (Fig. 1d). Increased minute ventilation resulted in decreased PaCO$_2$ and increased pH in patients treated with levosimendan (Table 2).

Table 2 also shows the absolute NME, ΔEA$_{di}$, $P_{0.1}$, C_{dyn}, PEEPi, respiratory rate, and inspiratory time over the course of the study.

Effects of levosimendan on hemodynamics
Levosimendan decreased mean arterial blood pressure (MAP) by 10% between the end of the CPAP trial before study medication ($T=0:30$) and start of the CPAP trial after study medication ($T=5:31$) (Fig. 2a, $P<0.001$). Compared to the placebo group, MAP in the levosimendan group was lower at the start of the CPAP trial after study medication ($T=5:31$) (MAP levosimendan 77 ± 3 and placebo 89 ± 4 mmHg; $P=0.031$).

In the levosimendan group, heart rate increased between start of administration of study medication ($T=1:00$) and start of the CPAP trial after study medication ($T=5:31$) (Fig. 2b, $P=0.046$).

Adverse events
No serious adverse events occurred between start of study protocol and 24 h after the end of the CPAP trial after study medication. Ten of 19 patients in the levosimendan group (one missing value due to technical reasons) developed hypotension (defined as systolic blood pressure <100 mmHg or a decrease $\geq20\%$) compared to 6 patients in the placebo group ($P=0.19$). Five patients

**Table 2 Respiratory and blood gas variables before and after study medication**

	Study time (hours:minutes)	Placebo (n = 19)				Levosimendan (n = 20)				P value		
		0:01	0:05	0:15	0:30	0:01	0:05	0:15	0:30	Time	Group	Interaction
NME (cmH$_2$O/μV)%	Before	1.8±0.6	1.8±0.5	1.7±0.6	1.7±0.5	1.2±0.2	1.3±0.3	1.3±0.3	1.2±0.2	0.84	0.71	0.41
	After +5:30	2.1±0.8	2.2±0.9	2.2±0.8	2.1±0.8	1.0±0.2	1.1±0.2	1.2±0.2	1.1±0.2			
EA$_{di}$ (μV)%	Before	15.2±2.7	17.6±3.4	17.6±3.2	18.0±3.3	15.8±2.8	16.0±2.7	16.3±2.8	16.2±2.8	0.02	0.93	0.38
	After +5:30	21.1±4.4	21.9±4.4	21.0±4.5	22.6±4.7*	19.8±3.6	20.6±3.6	19.3±3.7	21.1±4.0*			
P$_{0.1}$ (cmH$_2$O)%	Before	2.0±0.3	2.3±0.5	2.1±0.4	2.4±0.4	2.2±0.3	2.2±0.3	2.2±0.2	2.2±0.3	0.02	0.42	0.74
	After +5:30	3.2±0.6	3.2±0.6	3.1±0.6	3.2±0.6*	2.2±0.2	2.7±0.3	2.6±0.3	2.7±0.3*			
C$_{dyn}$ (mL/cmH$_2$O)%	Before	48.9±7.6	50.8±9.6	45.7±7.0	45.6±8.2	56.9±9.1	53.1±6.9	52.3±7.1	49.9±5.8	0.02	0.50	0.66
	After +5:30	41.5±7.9	41.5±8.1	43.9±11.0	48.0±13.1*	53.6±8.6	46.8±5.5	43.0±3.9	42.9±3.8*			
PEEPi (cmH$_2$O)%	Before	2.4±0.4	2.4±0.6	2.7±0.6	2.5±0.5	1.6±0.2	1.7±0.2	1.6±0.2	1.8±0.2	0.02	0.25	0.65
	After +5:30	3.1±0.6	3.0±0.6	2.9±0.5	3.1±0.6*	1.7±0.2	2.1±0.2	2.2±0.4	2.3±0.3*			
RR (breaths/minute)	Before	25±2	25±2	25±2	25±2	25±2	25±2	24±2	24±2	<0.01	0.91	0.38
	After +5:30	26±2	26±2	26±2	27±2*	26±2	26±2	27±2	26±2*			
T$_I$ (s)	Before	0.94±0.06	0.93±0.06	0.93±0.06	0.91±0.06	0.89±0.04	0.90±0.05	0.91±0.05	0.90±0.05	0.03	0.88	0.649
	After +5:30	0.90±0.06	0.89±0.06	0.90±0.06	0.89±0.07*	0.92±0.07	0.87±0.05	0.86±0.05	0.86±0.05*			
pH	Before	7.44±0.01	–	–	7.43±0.01	7.44±0.01	–	–	7.42±0.01	0.04	0.70	0.71
	After +5:30	7.44±0.01	–	–	7.44±0.01*	7.44±0.01	–	–	7.43±0.01*			
PaO$_2$ (mmHg)	Before	89±3	–	–	88±3	103±7	–	–	101±3	0.50	<0.01	0.56
	After +5:30	94±4	–	–	85±3	100±5	–	–	104±5			
PaCO$_2$ (mmHg)	Before	47±3	–	–	48±3	43±3	–	–	44±3	0.03	0.15	0.37
	After +5:30	47±2	–	–	48±3*	41±3	–	–	42±3*			

Data shown are averaged. Data are presented as mean ± SEM

CPAP continuous positive airway pressure, NME neuromechanical efficiency, EA$_{di}$ electrical activity of the diaphragm, P$_{0.1}$ decrease in esophageal pressure during the first 100 ms, C$_{dyn}$ dynamic lung compliance, PEEPi intrinsic positive end-expiratory pressure, RR respiratory rate, T$_I$ inspiratory time

*P < 0.05 compared to CPAP trial before study medication

% Parameters could only be determined in 18 patients in the placebo and 19 patients in the levosimendan group
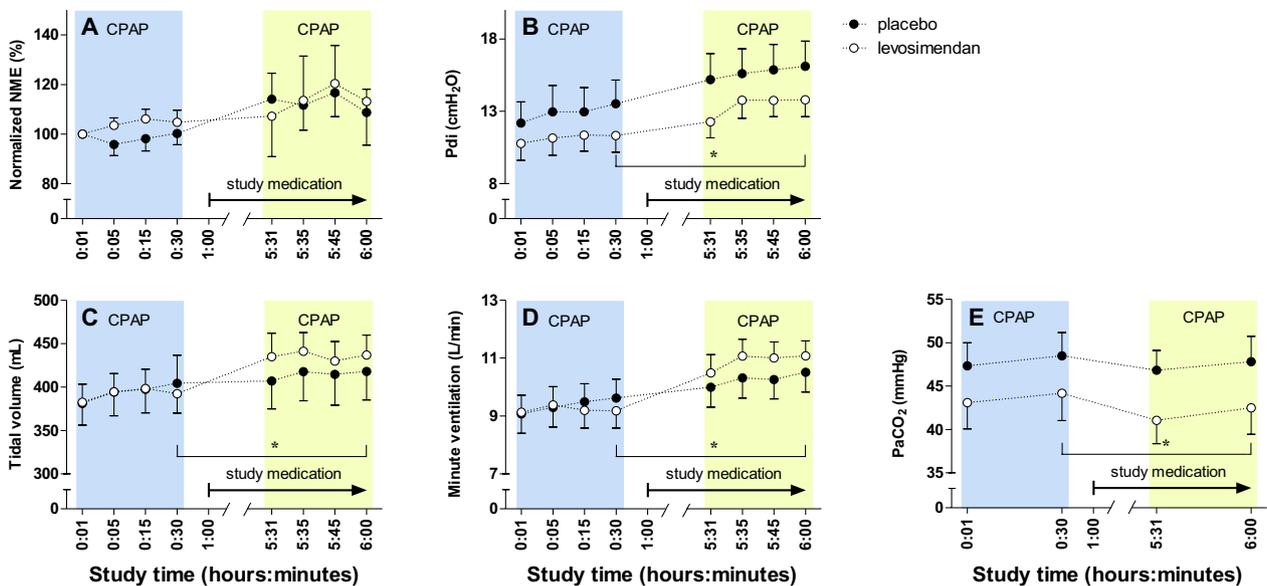
Fig. 1 Respiratory parameters over the course of the study day during the continuous positive airway pressure (CPAP) trial before (blue) and after (yellow) study medication for the placebo (closed circles) and levosimendan (open circles) groups. **a** Neuromechanical efficiency (NME) was normalized to the start point of the first CPAP trial [study time (hours:minutes) $T = 0:01$]. NME was not different between the placebo and levosimendan groups and did not increase in the continuous positive airway pressure (CPAP) trial after administration of levosimendan (primary outcome; $P = 0.407$). **b** Transdiaphragmatic pressure (ΔP_{di}) increased during the study day and was higher in the placebo and levosimendan groups in the CPAP trial after study medication as compared to the CPAP trial before study medication ($P < 0.001$). **c** Tidal volume increased during the study day in both groups, but increased significantly more after levosimendan administration as compared to the placebo group and as compared to the CPAP trial before study medication ($P = 0.017$). The same was true for minute ventilation (**d**), which also increased in both groups during the study day, but significantly more after levosimendan administration ($P = 0.021$). **e** $PaCO_2$ was higher in both groups in the CPAP trial after study medication as compared to the CPAP trial before study medication ($P = 0.032$). Tidal volume and minute ventilation could be determined in all patients ($n = 39$). The other parameters could be determined in 36 patients on time points, minute 1 and 5 during the CPAP trial before study medication ($T = 0:01$ and $T = 0:05$) and in 37 patients on the remaining time points. Data are presented as mean $\pm$ SEM. *Significantly different between CPAP trials
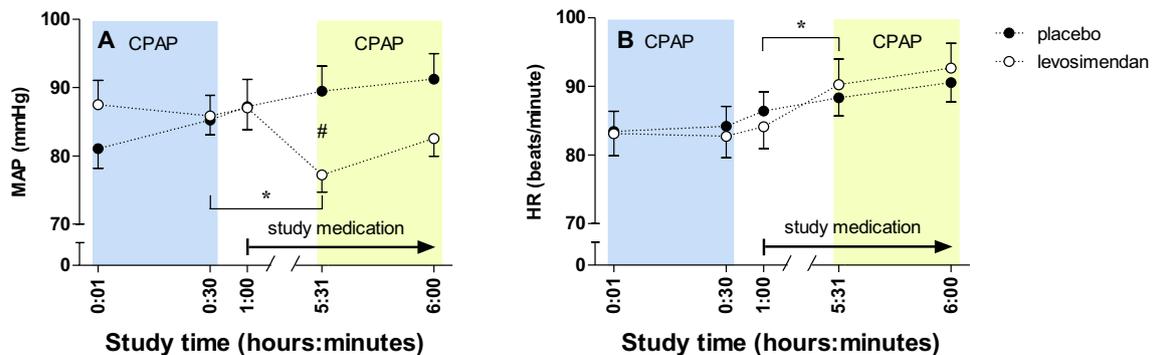
Fig. 2 Hemodynamic parameters during the study in the placebo (closed circles) and levosimendan groups (open circles). **a** Mean arterial blood pressure (MAP) was reduced at the start of the continuous positive airway pressure (CPAP) trial after study medication [study time (hours:minutes) $T = 5:31$] compared to the end of the CPAP trial before study mediation ($T = 0:30$) only in the levosimendan group ($P < 0.001$), in addition, MAP at the start of the CPAP trial after study medication ($T = 5:31$) was lower in the levosimendan group compared to the placebo group ($P = 0.031$). **b** In both groups, heart rate increased during the study day ($P < 0.001$). Only in the levosimendan group, heart rate was higher at the start of the CPAP trial after study medication ($T = 5:31$) compared to the start of study medication ($T = 1:00$) ($P = 0.046$). Data are presented as mean $\pm$ SEM (placebo $n = 19$, levosimendan $n = 20$). *Significantly different between time points in levosimendan group, #Significantly different between levosimendan and placebo group

required volume therapy after levosimendan administration compared to one patient in the placebo group ($P=0.18$).

Discussion

This is the first study to investigate the effects of levosimendan on diaphragm function in mechanically ventilated ICU patients. The main findings of this physiological double-blind randomized placebo-controlled trial can be summarized as follows: first, we found no effect of levosimendan on diaphragm contractile efficiency. These findings are partly unexpected, given our previous studies that demonstrated that levosimendan improves in vitro contractile efficiency of the diaphragm in elective surgery patients and patients with COPD [17], and also in vivo function of the diaphragm in healthy subjects [18]. Second, administration of levosimendan increased tidal volume, increased minute ventilation, and decreased $PaCO_2$. Third, no severe adverse events were reported after the administration of levosimendan.

Effects of levosimendan on diaphragm function

Critical-illness-associated respiratory muscle weakness may result from loss of contractile proteins (atrophy) or contractile protein dysfunction [5]. Indeed, only a few hours of controlled mechanical ventilation are associated with diaphragm atrophy in brain-dead patients [9, 13] and in patients after elective surgery [9]. More recently, we reported the development of atrophy in diaphragm muscle fibers of critically ill ventilated patients [12]. Using ultrasound, Goligher et al. [8] demonstrated progressive loss of diaphragm muscle thickness in ventilated ICU patients with high inspiratory ventilator assist. Besides atrophy, diaphragm weakness in critically ill patients results from dysfunction of the remaining contractile proteins, especially reduced calcium sensitivity of force generation [12, 14]. Reduced calcium sensitivity implies that more calcium and thus neural input is required to generate force, which increases energy expenditure of muscle contraction.

Levosimendan is known as a cardiac inotrope, with vasodilating properties. On one hand, it acts by increasing the sensitivity of troponin C to calcium in myocardial fibers, hence leading to inotropy. In addition, levosimendan increases open probability of the mitochondrial adenosine triphosphate (ATP)-sensitive potassium channels in smooth muscle cells, thus resulting in vasodilation (for review, see [25]). Levosimendan is currently approved as an inotrope for acutely decompensated heart failure [15, 16, 25]. In recent years, we have provided convincing evidence that levosimendan improves contractility of the diaphragm [17, 18, 26]. For instance, in human diaphragm muscle fibers,

levosimendan increases calcium sensitivity of contraction by 20–30% [17], which is expected to improve contractile efficiency. We have also demonstrated that the effects of levosimendan are more pronounced in slow-type fibers compared to fast-type fibers [17]. It should be noted that levosimendan does not increase maximum force-generating capacity of the muscle fibers, as maximum force is independent from calcium sensitivity at saturating calcium concentration. In vivo, levosimendan improved neuromechanical efficiency of the diaphragm by ~21% during both unloaded and loaded breathing [18]. Moreover, levosimendan reversed the development of diaphragm fatigue after strenuous loaded breathing. Based on these encouraging results in vitro (increased calcium sensitivity of contraction) and in vivo (improved neuromechanical efficiency of the diaphragm), we designed the current study. In the current study, measurements were performed during a CPAP trial, because at the time the study was designed, a CPAP trial was a common type of weaning trial. We found that levosimendan did not improve contractile efficiency of the diaphragm in ventilated critically ill patients compared to placebo. However, we did observe a significant increase in tidal volume and minute ventilation in the levosimendan group compared to placebo, resulting in a decrease in $PaCO_2$. Despite not being the primary endpoint, this outcome is clinically relevant as minute ventilation is the final common pathway of the respiratory system and sufficient minute ventilation is the key to successful weaning. The physiological mechanism for the increased minute ventilation remains speculative. First, these findings may be explained by an increase in central respiratory drive. As respiratory drive of the diaphragm (assessed by EA_{di}) was not different after levosimendan, it can only be explained by altered drive to other muscles of the respiratory muscle pump, such as accessory inspiratory muscles or expiratory muscles [27]. Second, improved cardiac function may decrease pulmonary congestion and thus improve respiratory mechanics. However, dynamic lung compliance was not affected by levosimendan, making this explanation less likely.

Interestingly, both in placebo and levosimendan groups, the EA_{di} and P_{di} increased in the second CPAP trial. Most likely, this reflects "normal" changes in respiratory drive during the day in ICU patients. As the first CPAP trial was performed early in the morning and the second in the afternoon, patients may have had some residual effects of sleep medication during the initial trial.

It should be noted that we expected that respiratory muscle function would improve in response to levosimendan regardless whether low levels of pressure support, CPAP or T piece with oxygen supply were used,

because in healthy subjects, we found improved respiratory muscle function in response to levosimendan during both unloaded and loaded breathing [18].

Explanations for unexpected findings

There are a number of possible explanations why levosimendan did not improve efficiency of the diaphragm in the current study. First, a too low dose of the study drug. In contrast to our previous study [18], we did not administer a loading dose before continuous infusion of levosimendan. This was chosen to decrease the risk of severe hypotension, especially because weaning patients are often treated with diuretics. Nevertheless, the total dose administered before measurements was higher in the current study compared to our study in healthy subjects [18] (3.8 mg compared to 3.0 mg, respectively). Indeed, levosimendan resulted in a decrease in blood pressure (Fig. 2a), consistent with its vasodilatory effects [15, 25]. In addition, the administered continuous dose was also based on previous sepsis trials in which equal dosages were administered [28]. Whether higher doses of levosimendan are effective to improve diaphragm function in ventilated ICU patients remains to be investigated. In the current study, half of the patients treated with levosimendan developed hypotension, half of them received fluid therapy. It is, therefore, expected, using higher doses of levosimendan, this will result in more pronounced hypotension in more patients.

Second, neuromechanical efficiency the diaphragm (NME; $\Delta P_{di}/\Delta EA_{di}$) was chosen as the primary endpoint to evaluate diaphragm function. NME reflects the pressure the respiratory muscles can generate for each microvolt of electrical activity. Previously, we demonstrated in healthy subjects that levosimendan improves NME of the diaphragm [18]. The validity of NME as outcome parameter depends on the quality of the P_{di} and EA_{di} signals. In the current study, mean NME values appear to be higher in the placebo group, this is, however, due to low EA_{di} in one patient, resulting in a high NME. A log transformation was applied prior to the two-way repeated measures ANOVA to obtain a normal distribution of the data. Recently, we reported that the EA_{di} processing algorithm of the ventilator software may limit the clinical applicability of NME [29, 30]. 'Non-physiological' EA_{di} curves may occur as a result of flaws in the processing algorithm (e.g., removal of ECG) (Fig. E1 in Online Supplement), which affect the NME as well. In contrast to the current study, we used raw diaphragm EMG in our in vivo study in healthy subjects [18]. Another notable difference between the current study and our study in healthy subjects is that the healthy subjects had to perform inspiratory maneuvers of 10 s against a closed valve, resulting in a stable diaphragm EMG signal and, therefore, less

vulnerable to artifacts as compared to the EA_{di} signal obtained during a single inspiration in the current study. Besides technical issues regarding the primary endpoint, levosimendan has effects beyond calcium sensitization, including modulation of mitochondrial function and vasodilation [25]. This could have affected respiratory muscle function, independent from effects on NME. In a recent randomized controlled trial in patients with sepsis [31], levosimendan did not affect organ dysfunction (SOFA score) or mortality, but increased time to successful weaning. It should be noted that levosimendan in that study was administered very early after ICU admission and not in the weaning phase. Therefore, that study does not preclude a beneficial effect of levosimendan on patients weaning from mechanical ventilation.

Third, future studies should also consider the use of other calcium sensitizers, despite our earlier encouraging findings with levosimendan [17, 18, 26]. Alternatively, fast skeletal troponin activation might improve respiratory muscle function. Tirasemtiv amplifies the force response of the sarcomeres to calcium by slowing its dissociation rate from the troponin complex in fast-twitch muscle fibers [32]. Hooijman et al. [14] demonstrated that CK-2066260, an analog of tirasemtiv, increases the calcium sensitivity and restored contractile force of fast diaphragmatic muscle fibers of critically ill patients. The effects of CK-2066260 on diaphragm contractility in vivo have not been evaluated. Recently, however, tirasemtiv failed in a Phase 3 clinical trial in patients with amyotrophic lateral sclerosis due to its crossing the blood–brain barrier [33].

Strengths and limitations

Previous studies demonstrating that levosimendan improves the calcium sensitivity of human diaphragm fibers in vitro [17, 26] and the contractile efficiency of the diaphragm in healthy subjects in vivo [18] provided a strong physiological rationale to perform the current study. In depth, physiological analysis of the diaphragm function was performed before and after study medication under controlled conditions (CPAP).

This study has some limitations. First, the endpoint, NME, has limited clinical relevance and the final effects of levosimendan on weaning success remain to be investigated. As the study protocol did not specifically describe criteria for extubation, reintubation, management for extubation failure, and so on, we did not report data on clinical outcome after the second CPAP trial. Second, we did not assess the effects of levosimendan on cardiac function, for instance, using cardiac ultrasound. We specifically excluded patients with past medical history of heart failure or current use of inotropes. Therefore, it was reasoned that changes in hemodynamic

condition due to levosimendan were unlikely to affect the primary endpoint. Third, we did not measure $P_{\text{di-twitch}}$, which is the gold standard to evaluate respiratory muscle function and can be measured upon magnetic or electrical stimulation of the phrenic nerves. We did not use this technique in the current study, because it can be technically challenging to perform, has limitations in tolerance, and cannot be applied in every patient [34]. Fourth, the calculated sample size was 38 patients. After finishing the study, it appeared that for only 37 patients the primary endpoint could be evaluated (one patient in each group was excluded). However, it seems unlikely that this would affect the primary outcome of the study.

In conclusion, this double-blind randomized placebo-controlled trial demonstrates that in patients weaning from mechanical ventilation, levosimendan failed to demonstrate a direct effect on diaphragm function, but does increase minute ventilation. The effects of levosimendan on weaning outcome remain to be studied.

Electronic supplementary material

The online version of this article (https://doi.org/10.1007/s00134-019-05767-y) contains supplementary material, which is available to authorized users.

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Author contributions

Study conception and design: JD, LH; data acquisition: LR, JD; data analysis: LR, JD, CS, LH; data interpretation: all authors; manuscript drafting and revising: all authors.

Compliance with ethical standards

Conflict of interest

Lisanne Roesthuis: Declares no conflict of interest. Hans van der Hoeven: Declares no conflict of interest. Christer Sinderby: Dr. Sinderby has made inventions related to neural control of mechanical ventilation that are patented. The patents are assigned to the academic institution(s) where inventions were made. The license for these patents belongs to Maquet Critical Care. Commercial uses of this technology may provide financial benefit to Dr. Sinderby through royalties. Dr Sinderby owns 50% of Neurovent Research Inc

(NVR). NVR is a research and development company that builds the equipment and catheters for research studies. Tim Frenzel: Declares no conflict of interest. Coen Ottenheijm: Declares no conflict of interest. Laurent Brochard: Laurent Brochard's laboratory has received grants and/or equipment for research projects from Medtronic Covidien, Philips, Sentec, Air Liquide, Fisher Paykel. Jonne Doorduin: Declares no conflict of interest. Leo Heunks: Has received speakers fee from Maquet Critical Care (Sweden) and Orion Pharma (Finland). His laboratory has received research grants a/o equipment from Liberate Medical (USA) and Maquet (Sweden).

Ethical approval

All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee (CMO Regio Arnhem-Nijmegen NL-number 40137.091.12) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

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