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Aggravation of myocardial dysfunction by injurious mechanical ventilation is mediated through a calcium-independent mechanism in LPS-induced pneumonia in rats

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Shock [submitted]

Abstract

We investigated the effects of injurious mechanical ventilation (MV) on myocardial function in a two-hit animal model of lipopolysaccharide (LPS)-induced pneumonia. We hypothesized that ventilation with injurious high tidal volume (V_t) induces ventilator-induced lung injury (VILI), and, by spill over of inflammatory mediators, induces myocardial inflammation and decreases myocardial function in a calcium-independent manner. Male rats treated with 2mg/kg LPS intratracheally or non-treated rats were ventilated with normal (6 ml/kg+5 cm H_2O positive end expiratory pressure (PEEP)) and injurious (19ml/kg+1cm H_2O PEEP) ventilation for 4 hours. High V_t ventilation induced VILI as oxygenation decreased by LPS and high V_t ventilation, mean airway pressure increased by high V_t ventilation suggesting increased lung stiffness, and pulmonary wet/dry ratio increased by LPS and high V_t ventilation. Myocardial function, measured *ex vivo* in isolated Langendorff-perfused hearts, decreased by LPS and this dysfunction was aggravated by high V_t ventilation. Calcium sensitivity was decreased by LPS but not altered by high V_t ventilation. Pulmonary heat shock protein (Hsp)70 mRNA expression was increased by the interaction between LPS and high V_t ventilation. Myocardial toll-like receptor (TLR)2 mRNA expression was increased by LPS. Myocardial chemokine (C-X-C motif) ligand (CXCL)1 mRNA expression was increased by LPS and was further increased after high V_t ventilation. In conclusion, mechanical ventilation aggravates LPS-induced myocardial dysfunction through a calcium-independent pathway. Induction of myocardial CXCL1 after binding of Hsp70, released by the lungs, to myocardial TLR2 may play a role.

Introduction

Injurious mechanical ventilation (MV) can induce ventilator-induced lung injury (VILI) and may thereby subsequently contribute to multiple organ failure, potentially by spill over from lung-borne inflammatory mediators into the circulation which may act on distant organs such as the kidneys (1-3). Furthermore, the harmful effects of VILI depend on the type of underlying lung injury. Direct or primary pulmonary injury, like pneumonia or aspiration, is associated with more severe injury when compared to indirect pulmonary injury, like sepsis, in animals exposed to identical ventilatory settings (4;5).

In contrast to the kidney, the effect of VILI on the heart is less clear. This can partly be explained by the fact that underlying diseases such as sepsis may hamper both pulmonary function (6), thereby creating need for MV, and myocardial function (7-10). Only a few studies have addressed the effect of MV on myocardial function. It was suggested that plasma from dogs ventilated with 15 cm H₂O positive end expiratory pressure (PEEP) contains a negative inotropic mediator (11) which may affect myocardial function. Next, Nin et al. showed that high tidal volume (V_t) ventilation induces VILI and upregulates myocardial cyclooxygenase (COX)-1 and COX-2, enzymes that are known to be up-regulated during inflammatory conditions (12). Also, Brander et al showed that high V_t ventilation during acute lung injury (ALI) induced VILI and increased myocardial interleukin (IL-) 8 expression, whereas this was decreased with so-called lung-protective strategies (13). These studies suggest that pulmonary overdistention and VILI may induce myocardial inflammation and adversely affect myocardial function.

In the current study we used a two hit model of lipopolysaccharide (LPS)-induced pneumonia and subsequent high V_t ventilation to induce VILI and studied myocardial function *ex vivo*. We hypothesized that spill over of inflammatory mediators induces myocardial inflammation and decreases myocardial function which may be adversely affected by LPS. Heat shock protein (Hsp)-70 may be induced in the lungs during VILI (14;15) and may, after spill over, bind to myocardial toll-like receptor (TLR)-2 (16). It may thereby induce an inflammatory response in the heart and decrease myocardial function through a calcium-independent pathway.

Materials and Methods

Animal experiments. All experiments applied with the Guide for Care and Use for laboratory animals of the National Institute of Health and were approved by the Institutional Animal Care and Use

Committee of the VU University Amsterdam. Thirty-two male Wistar rats weighing 330 ± 20 g were anesthetized with 12.5 mg/kg midazolam (Pharmachemie BV, Haarlem, the Netherlands) and 85 mg/kg ketamine (Alfasan, Woerden, the Netherlands) i.p. and 10 mg/kg ketamine i.m. Anesthesia was maintained with 1.2 mg/kg/h midazolam and 20 mg/kg/h ketamine i.v. Paralysis was maintained with pancuronium 0.6 mg/kg/h i.v. to allow for high Vt ventilation. Animals were placed in supine position on a heating pad maintaining body temperature at 37 °C. A tracheostomy was performed and a 14 Gauge canula was inserted into the trachea. During preparation, animals were ventilated with a Vt of 6 ml/kg and 5 cm H₂O positive end-expiratory pressure (PEEP) (Avea, CareFusion, Houten, the Netherlands). Catheters were inserted into the left carotid artery and left jugular vein for arterial blood sampling and continuous measurement of the mean arterial pressure (MAP) and central venous pressure (CVP) in mmHg. Heart rate (HR) in beats per minute (BPM) was derived from MAP measurements. The femoral artery was catheterized with a thermistor from a pulmonary artery catheter to measure cardiac output. Cardiac output was obtained every 30 minutes by averaging two successive thermodilution determinations (CO Computer, 9520A, Edwards laboratory, Santa Ana, Ca, USA), for which 200 µl of cold saline was injected via the right jugular vein catheter as described previously (17). Blood samples (150 µl) were taken every hour for gas analysis and these were replaced by equal volumes of normal saline. Blood gas analysis was performed using a pH blood-gas analyzer (ABL 50; Radiometer, Copenhagen, Denmark). Partial pressure of arterial oxygen (P_aO₂, mmHg)/fraction of inspired oxygen (F_IO₂, %) ratios were calculated.

Experimental protocol. After preparation, hemodynamics were allowed to stabilize for 10 min after which base line values were established (t=-10). At t=-5 animals were randomized to non-LPS treated or LPS treated groups. The latter received saline-dissolved LPS (2 mg/kg, LPS L2880, LPS from E. Coli 055:B5, Sigma-Aldrich) intratracheally using a miniature nebulizer (Penn-Century, Wyndmoor, PA, USA). Five minutes after LPS administration, the protocol was started and animals were randomly assigned to one of two ventilation strategies; ventilation with either low Vt (Vt 6 ml/kg, 5 cm H₂O PEEP) which is regarded as a lung protective strategy (18), or high Vt (Vt 19 ml/kg, 1 cm H₂O PEEP) which is regarded as an injurious strategy. Ventilatory settings were chosen such that mean airway pressure was similar. Thus four groups were studied; non-LPS treated (n=8) and LPS-treated animals (n=8) ventilated with low tidal volume and non-LPS treated (n=11) and LPS-treated animals (n=8) ventilated with high tidal volume. The F_IO₂ was set at 0.4 in both groups but was increased when oxygenation was impaired. Ventilation rate was set to maintain normocapnia. Four hours after the

start of the protocol hearts were rapidly dissected and myocardial function was measured *ex vivo*. If P_aO_2/F_iO_2 fell below 150 with a F_iO_2 of 100% and an increase in mean airway pressure of 2 cm H₂O compared to t=0 was present, the experiment was terminated prematurely and myocardial function *ex vivo* was measured.

Myocardial function ex vivo. Myocardial function *ex vivo* was measured in a Langendorff set-up (n=8 per group), to study myocardial function independent of loading condition as previously described (19). Briefly, the aorta of the isolated heart was cannulated and the heart was perfused with a modified Krebs-Henseleit solution at a constant coronary perfusion pressure of 80 mmHg at 37 °C. The modified Krebs-Henseleit solution contained (in mM) 118.5 NaCl, 4.7 KCl, 25 NaHCO₃, 1.2 MgCl₂, 1.2 KH₂PO₄ and 11 glucose and was equilibrated with 95% O₂ and 5% CO₂ at a pH of 7.4. Solutions with different CaCl₂·2(H₂O) concentrations were made, resulting in final calcium concentrations of 0.5; 1; 2 and 4 mM. Both right and left atria were removed and hearts were paced at 5 Hz with electrodes. Afferent coronary flow was measured with a flow meter (Transonic Systems Europe B.V., Maastricht, the Netherlands). A custom-made balloon was inserted in the left ventricle to measure isovolumic pressures with a catheter tip manometer system (19) and the heart was allowed to stabilize for 10 minutes. Ventricular volume at maximal pressure development (V_{max}) was determined and balloons were adjusted to 85% of V_{max} . Hearts were allowed to stabilize for 2 min. After stabilization, myocardial function was measured by systolic and diastolic pressure, maximal rates of pressure development ($+dP/dt_{max}$) and pressure decline ($-dP/dt_{min}$). As measurements were performed at a fixed heart rate and preload, $+dP/dt_{max}$ and $-dP/dt_{min}$ can be regarded as indices of contractility and relaxation, respectively (7). Developed pressure was calculated as systolic pressure minus diastolic pressure. Force-pCa (i.e. $-\log_{10}[Ca^{2+}]$) relations were fit to the Hill equation and subsequently the pCa₅₀, the pCa at which 50% of the developed force was reached, was calculated (20). A decrease in the pCa₅₀ indicates a decrease in Ca²⁺ sensitivity. After this protocol, hearts were removed from the isolated Langendorff-perfused heart set-up, cut transversally in three sections, frozen in liquid nitrogen and stored at -80 °C. The apical section of the heart was used to calculate wet to dry weight ratio.

Wet to dry weight ratios. Immediately after the animals were sacrificed, middle right lung lob was taken and weighed, dried at 37 °C and weighed again. After freezing, apical section of the heart was weighed, freeze-dried and weighed again.

mRNA expression. To study myocardial inflammatory response we studied myocardial mRNA expression of the key pro-inflammatory mediators TNF α , interleukin (IL)-6, IL-1 β , chemokine (C-X-C

motif) ligand (CXCL)-1 and TLR-2. These mediators were chosen as their expression was shown to either correlate with HSP70 expression (14) or be induced by binding of Hsp70 to TLR2 (16). Myocardial tissue from the center transversal section and pulmonary tissue from the right lung were pulverized with a mortar and RNA was extracted using Trizol. Pulmonary RNA was purified using the RNA clean up kit (Qiagen, Venlo, the Netherlands). DNA was removed by DNase I amplification grade (Invitrogen, Breda, the Netherlands) A total of 2 µg RNA was used to synthesize copy DNA (cDNA) using a Cloned AMV First Strand cDNA Synthesis Kit (Invitrogen, Breda, The Netherlands) using oligo-dT priming. Quantitative RT-PCR analysis was performed using SYBR Green in an ABI 7500 sequence detection system (Applied Biosystems, Foster City, USA). Briefly, 8 µl mix was prepared using 25 ng cDNA, forward and reverse primers and Mesa Green qPCR Mastermix Plus for SYBR assay (Eurogentec, Maastricht, the Netherlands). The used protocol was 2 min 50°C, 10 min 95°C, 40 cycles (0:15 min 95°C, 1:00 min 58°C) and a dissociation curve. Primer sequences are shown in table 1. Cycle threshold values (Ct), the number of cycles required for the fluorescent signal to cross the threshold, were measured. Relative expression levels of target genes were calculated relative to the housekeeping gene β-actin with the formula $2^{-(Ct(\beta\text{-actin}) - Ct(\text{target gene}))}$.

Statistical analysis. To analyze the effects of LPS, Vt and their interaction, general estimated equations (GEE) were performed. With GEE effects of separate parameters as well as their interaction can be calculated, as in two-way ANOVA, taking repeated measures over time in the same animals and baseline values as covariates into account when appropriate. A statistically significant interaction implies that the effect of high Vt ventilation over time differs among the non-LPS and LPS-treated animals. T-tests were performed to evaluate the effect of high Vt ventilation in LPS-treated animals on mRNA expression. Spearman correlations were calculated when non-parametric distributed parameters were tested. Data are shown as mean ± SEM, exact P values are given if >0.001. A P-value < 0.05 was considered statistically significant.

Results

Baseline characteristics were similar between the groups. Seven LPS-treated animals ventilated with high Vt died prematurely (4 after t=150 min; 1 after t=180 min; 2 after t=210 min).

Hemodynamic measurements. Hemodynamic data are shown in Figure 1. MAP (P<0.001), CVP (P<0.001) and HR (P<0.001) decreased by high Vt ventilation, but no effect of LPS was seen. Cardiac output decreased by the interaction between high Vt ventilation and LPS (P=0.001). Blood pH

HSP-70	Forward	AGGTGGATTAGAGGCTCTTT
	Reverse	AACCTAGGACTTGATTGCAGA
TNF- α	Forward	ACAAGCCCGTAGCCACGTC
	Reverse	AGGAGCACGTAGTCGGGGCA
IL-6	Forward	GTCTCGAGCCCACCAGGAACG
	Reverse	AAGCCTCCGACTTGTGAAGTGGT
IL-1 β	Forward	GAGCCCGTCCTCTGTGACTCGT
	Reverse	AGGCCCAAGGCCACAGGGATT
CXCL-1	Forward	TCGCCAATGAGCTGCGCTGT
	Reverse	CAAGGCAAGCCTCGCGACCAT
TLR-2	Forward	GGGAAGGCCATTCTGCCAGG
	Reverse	CGGAGGTTACACAGGCTCGC
β -actin	Forward	GGCCAACCGTGAAAAGATGA
	Reverse	GGACAACACAGCCTGGATGG

Table 1. Primer sequences used for RT-PCR

decreased by LPS (Figure 2a, $P < 0.001$) but only LPS-treated animals ventilated with high V_t reached levels consistent with acidosis.

Respiratory parameters. PCO_2 increased by LPS ($P < 0.001$) and decreased by high V_t ventilation (Figure 2b, $P < 0.001$) but remained within the normal range in all cases. P_aO_2/F_iO_2 decreased by both LPS ($P = 0.022$) and high V_t ventilation (Figure 2c, $P = 0.011$). Mean airway pressure slightly increased by high V_t ventilation (Figure 2d, $P < 0.001$) but was not affected by LPS. Pulmonary wet/dry ratio increased by both LPS ($P < 0.001$) and high V_t ventilation (Figure 2e, $P < 0.001$) and an interaction was seen ($P = 0.001$). Pulmonary Hsp-70 mRNA expression increased by the interaction between LPS and high V_t ventilation (Table 2, $P < 0.001$).

Myocardial function ex vivo. LPS decreased myocardial contractile function *ex vivo* as can be seen by a decrease in LV systolic pressure (Figure 3a, $P < 0.001$), LV developed pressure (Figure 3c, $P = 0.004$) and LV $+dP/dt_{max}$ (Figure 3d, $P = 0.015$). LPS also decreased relaxation as can be seen by an increase in LV $-dP/dt_{min}$ (Figure 3e, $P = 0.001$). An interaction of LPS with high V_t ventilation was seen for LV developed pressure ($P = 0.006$), LV $+dP/dt_{max}$ ($P = 0.006$) and LV $-dP/dt_{min}$ ($P = 0.007$). Cardiac pCa_{50} decreased by LPS (Figure 3f, $P < 0.001$) but was not affected by high V_t ventilation. Affluent coronary flow (16.0 ± 1.1 ml/min) and cardiac wet/dry ratio (6.6 ± 0.1) were similar between the groups.

Myocardial inflammation. TLR-2 mRNA expression increased by LPS ($P = 0.035$) but not by high V_t

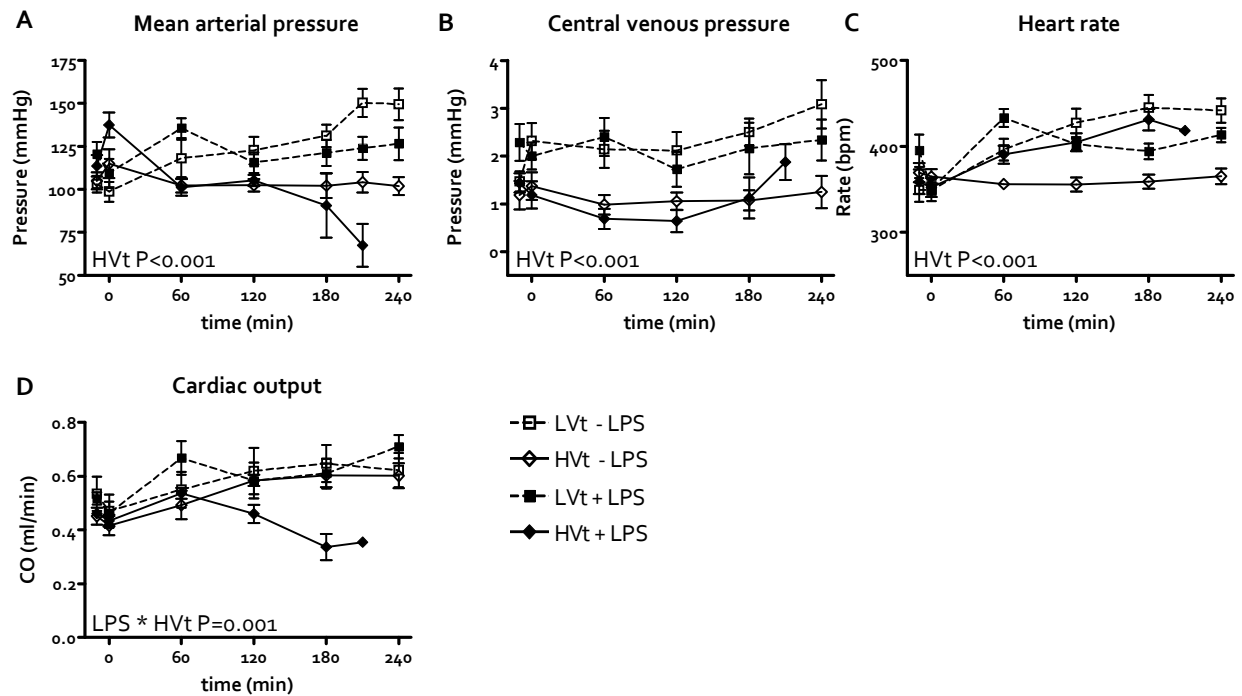


Figure 1. *In vivo* measurements in the course of time (LPS=lipopolysaccharide; LVt=low tidal volume (Vt) ventilation, HVt=high Vt ventilation). Mean arterial pressure (A), central venous pressure (B) and heart rate (C) decreased by high Vt ventilation ($P < 0.001$). D. Cardiac output decreased by the interaction between LPS installation and high Vt ventilation ($P = 0.001$).

ventilation. Myocardial CXCL-1 mRNA expression increased by LPS ($P = 0.017$), high Vt ventilation ($P = 0.004$) and an interaction between LPS and high Vt was seen ($P = 0.013$). CXCL-1 ($r = -0.60$, $P = 0.002$) and TLR-2 ($r = -0.54$, $P = 0.008$) mRNA expressions correlated inversely with LV systolic pressure. TLR-2 mRNA expression correlated with LV $-dp/dt_{min}$ ($r = 0.48$, $P = 0.021$). Myocardial TNF- α , IL-6 and IL-1 β mRNA expressions were not altered by either LPS or high Vt ventilation.

Discussion

In this study, we show that injurious MV with high Vt aggravates LPS-induced myocardial dysfunction *ex vivo* through a calcium-independent pathway in a model of LPS-induced pneumonia.

We used a two-hit model with intratracheal LPS installation and MV with Vt of 19ml/kg to create VILI (21;22). Our model was rather severe, as only one animal treated with LPS and high Vt ventilation survived 4 hours. High Vt ventilation increased mean airway pressure, suggesting increased pulmonary stiffness, and intratracheal LPS installation with high Vt ventilation impaired gas exchange and increased pulmonary wet/dry ratio, which is consistent with VILI (23). High Vt ventilation decreased MAP, CVP and HR in all animals (24) and decreased CO in LPS-treated animals. At the end

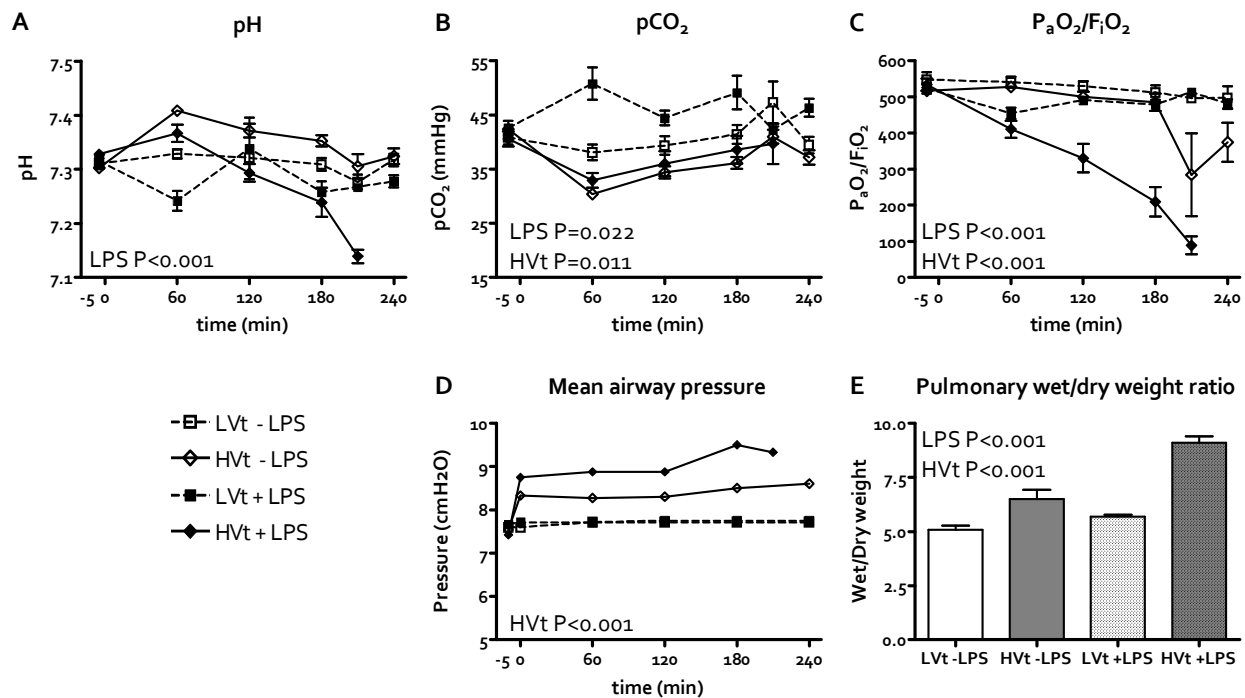


Figure 2. VILI induction in the course of time (LPS=lipopolysaccharide; LVt=low tidal volume (Vt) ventilation, HVt=high Vt ventilation, P_aO₂= partial pressure of oxygen in arterial blood, F_iO₂= fraction of inspired oxygen). A. pH decreased by LPS installation (P<0.001). B. P_aO₂/F_iO₂ ratio decreased by LPS installation (P=0.022) and high Vt ventilation (P=0.011). C. PCO₂ increased by LPS installation (P<0.001), and decreased by high Vt ventilation (P<0.001). D. Mean airway pressure increased by high Vt ventilation (P<0.001). E. Pulmonary wet/dry ratio increased both by LPS (P<0.001) and high Vt ventilation (P<0.001).

of the experiment, CVP increased in LPS-treated animals ventilated with high Vt but this did not reach significance probably as a result of the premature deaths in this group. These data suggest a decreased cardiac work in animals ventilated with high Vt particularly in LPS-treated animals. Moreover, the increased CVP together with decreases in MAP and CO in LPS-treated animals ventilated with high Vt suggest myocardial failure at the end of the experiment.

In line with previous studies, myocardial function *ex vivo* decreased after LPS exposure (8). The LPS-induced myocardial dysfunction *ex vivo* was aggravated by high Vt ventilation as shown by a further decrease in LV developed pressure, LV +dP/dt_{max} and LV -dP/dt_{min}. As coronary flow *ex vivo* was similar between the groups, observed differences in myocardial function were not caused by differences herein. Myocardial edema may affect myocardial function (25) but no difference in cardiac wet/dry ratio was found, thereby excluding edema as a causative factor. Furthermore, it was postulated that distant organ failure after injurious MV might be due to spill over of LPS from the injured lungs into the systemic circulation (26;27) which can then decrease myocardial function by, amongst others, altering myocardial calcium handling and sensitivity (9;10). Indeed, in our study LPS-

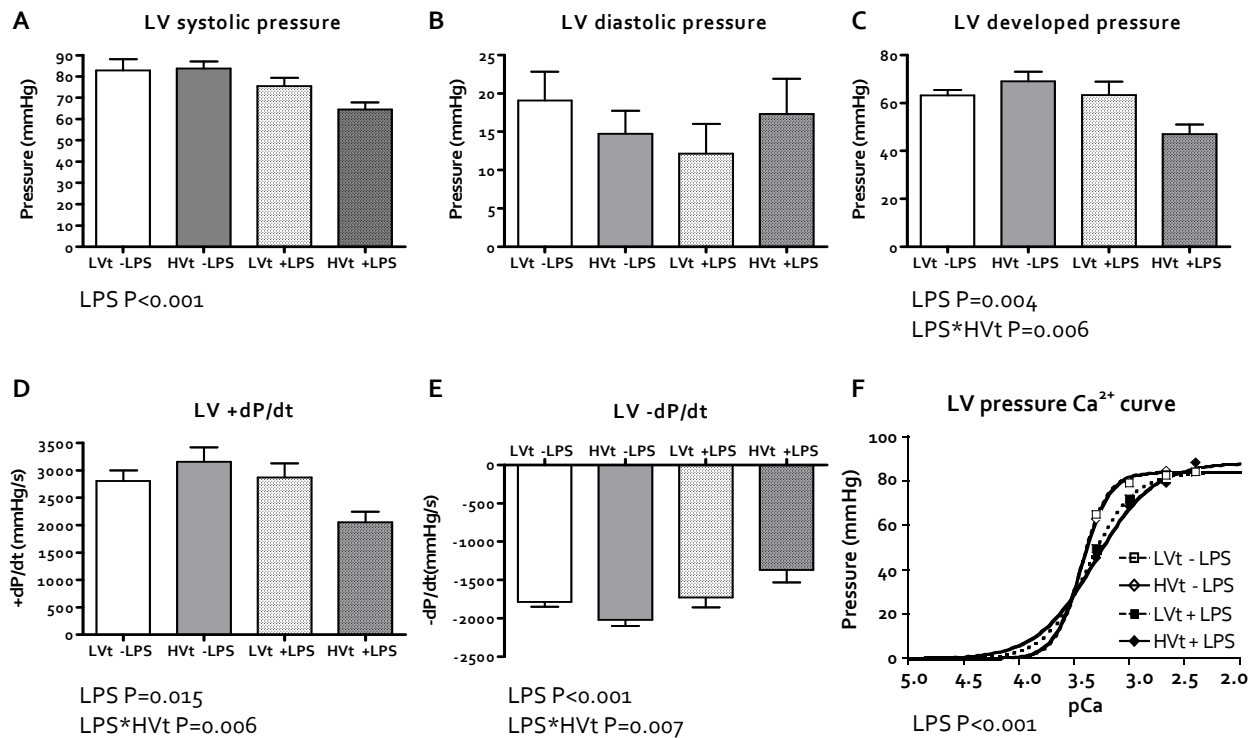


Figure 3. Myocardial function ex vivo (LPS=lipopolysaccharide; LVt=low tidal volume (Vt) ventilation, HVt=high Vt ventilation). A. Left ventricular (LV) systolic pressure decreased by LPS installation ($P < 0.001$). B. LV diastolic pressure was not altered by either LPS or high Vt ventilation. C. LV developed pressure decreased by LPS installation ($P = 0.004$) and the interaction between LPS installation and high Vt ventilation ($P = 0.006$). D. LV contractility, measured as $+dP/dt_{max}$, decreased by LPS installation ($P = 0.015$) and the interaction between LPS installation and high Vt ventilation ($P = 0.006$). E. LV relaxation, measured as $-dP/dt_{min}$, decreased by LPS installation ($P < 0.001$) and the interaction between LPS installation and high Vt ventilation ($P = 0.007$). F. LV pCa_{50} decreased by LPS ($P < 0.001$)

induced myocardial dysfunction was associated with decreased cardiac pCa_{50} , but high Vt ventilation did not affect cardiac pCa_{50} . These results suggest that the high Vt ventilation-induced aggravation of LPS-induced myocardial dysfunction was not due to LPS spill over but was mediated by another, calcium-independent, mechanism. Similarly, as acidosis may decrease myocardial function through a calcium-dependent mechanism (28), acidosis is less likely to be a cause of the observed aggravated dysfunction. To the best of our knowledge, this is the first study in which myocardial function during VILI is measured independent of loading differences. Although it was suggested that myocardial function is decreased during VILI by similar mechanisms as during sepsis (29), our study suggests that other mechanisms may play a role.

As myocardial function decreased with increasing VILI, a factor related to the severity VILI must be involved. We found that pulmonary Hsp-70 mRNA expression increased in severe VILI, after high Vt ventilation in combination with LPS exposure. Although we cannot explain the Hsp-70 mRNA

	LV _t -LPS	HV _t -LPS	LV _t +LPS	HV _t +LPS
Pulmonary mRNA expression (A.U.)				
Hsp-70 <i>LPS*HV_t; P<0.001</i>	17*10 ⁻³ (8.7*10 ⁻³ -26*10 ⁻³)	2.5*10 ⁻³ (0.050*10 ⁻³ -8.2*10 ⁻³)	3.8*10 ⁻³ (3.4*10 ⁻³ -6.2*10 ⁻³)	16*10 ⁻³ (8.4*10 ⁻³ -27*10 ⁻³)
	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6	<i>n</i> =5
Myocardial mRNA expression (A.U.)				
TLR-2 <i>LPS; P=0.035</i>	0.10*10 ⁻³ (0.0-0.22*10 ⁻³)	0.10*10 ⁻³ (0.0-0.25*10 ⁻³)	0.10*10 ⁻³ (0.10*10 ⁻³ -0.25*10 ⁻³)	0.25*10 ⁻³ (0.18*10 ⁻³ -0.55*10 ⁻³)
	<i>n</i> =6	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6
TNF-α	0.10*10 ⁻³ (0.0-0.38*10 ⁻³)	0.15*10 ⁻³ (0.075*10 ⁻³ -1.6*10 ⁻³)	0.20*10 ⁻³ (0.05*10 ⁻³ -0.35*10 ⁻³)	0.45*10 ⁻³ (0.10*10 ⁻³ -0.63*10 ⁻³)
	<i>n</i> =6	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6
IL-6	0.0 (0.0-0.35*10 ⁻³)	0.050*10 ⁻³ (0.0-3.1*10 ⁻³)	0.10*10 ⁻³ (0.0-7.0*10 ⁻³)	0.35*10 ⁻³ (0.0-4.7*10 ⁻³)
	<i>n</i> =6	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6
IL-1β	0.45*10 ⁻³ (0.075*10 ⁻³ -3.9*10 ⁻³)	1.3*10 ⁻³ (0.38*10 ⁻³ -15*10 ⁻³)	1.6*10 ⁻³ (0.65*10 ⁻³ -2.6*10 ⁻³)	1.9*10 ⁻³ (0.83*10 ⁻³ -4.9*10 ⁻³)
	<i>n</i> =6	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6
CXCL-1 <i>LPS; P=0.017</i> <i>HV_t; P=0.004</i> <i>LPS*HV_t; P=0.013</i>	17*10 ⁻³ (2.7*10 ⁻³ -57.8*10 ⁻³)	12*10 ⁻³ (3.8*10 ⁻³ -67*10 ⁻³)	21*10 ⁻³ (16*10 ⁻³ -37*10 ⁻³)	119*10 ⁻³ (71*10 ⁻³ -225*10 ⁻³)
	<i>n</i> =6	<i>n</i> =6	<i>n</i> =5	<i>n</i> =6

Table 2. Pulmonary and myocardial mRNA expression in arbitrary units (A.U.). Relative expression levels of target genes were calculated relative to the housekeeping gene β -actin with the formula $2^{(Ct(\beta\text{-actin})-Ct(\text{target gene}))}$. Median values and interquartile ranges are shown. (LPS=lipopolysaccharide; LV_t=low tidal volume (Vt) ventilation, HV_t=high Vt ventilation, HSP70=heat shock protein 70, TLR2=toll like receptor 2, TNF α=tumor necrosis factor α, IL6=Interleukin 6; IL1β=Interleukin 1β, CXCL1=chemokine (C-X-C motif) ligand 1)

expression in the animals with low Vt ventilation without LPS, an increase in Hsp-70 after high Vt ventilation and LPS-expression was shown before (14;15). We did not measure serum Hsp-70 concentrations but it was shown before that Hsp70 may be actively released by cells under stress (30). Indeed, injuring the lung with diesel exhaust, caused increases in both pulmonary and systemic Hsp-70 expression (31) Extracellular Hsp-70 can bind to myocardial TLR-2 and may thereby cause calcium-independent myocardial dysfunction and an inflammatory response shown by, amongst others, an increase in myocardial CXCL-1 expression (16). Indeed, in this study we found that VILI-associated myocardial dysfunction was mediated through a calcium-independent mechanism. We found a LPS-induced upregulation of myocardial TLR2 in agreement with others (32), so that the myocardium of

LPS-treated animals were more prone to bind extracellular Hsp70 spilled over from the lungs. Mathur et al. found a TLR2-mediated increase in CXCL1 after stimulating cardiomyocytes with extracellular Hsp70 (16). We also found induction of CXCL1 after LPS and even more so after high Vt ventilation and this CXCL1 mRNA expression correlated inversely with LV systolic pressure. Although the direct effect of CXCL1 on cardiac function is unknown, its expression was shown to be upregulated in myocardial inflammatory states (16;33) and inhibition of CXCL1 decreased right ventricular failure in a model of pulmonary embolism (34). Expression of other inflammatory mediators was not increased, which might be due to a different time course of expression. We have previously shown that myocardial TNF α expression is increased 2 hours after LPS exposure, but not detectable after 4 hours (8). Based on these results, we suggest that during lung injury, induced by LPS installation with subsequent high Vt ventilation, Hsp70 is produced in the lungs which may spill over into the circulation and bind to the TLR2 on the myocardium, whose expression was increased after LPS. Thereby it may cause a VILI-induced calcium-independent aggravation of myocardial dysfunction, potentially mediated through CXCL1. However, this concept warrants further investigation by intervention studies.

As we measured calcium sensitivity in hearts with intact cell membranes, the calcium sensitivity in this study should not be confused with myofilament sensitivity (20). As we cannot distinguish between myofilament sensitivity and calcium handling we cannot exclude the possibility that both parameters were altered. However, as the net result of these parameters did not show a difference in overall calcium sensitivity, we may conclude that the VILI-induced aggravation of myocardial dysfunction is mediated by a calcium-independent pathway.

In conclusion, our study shows that severe VILI after injurious MV during LPS-induced pneumonia can aggravate myocardial dysfunction, and that this effect is dependent on the severity of VILI. Whereas it was previously suggested that VILI may affect myocardial function through similar pathways as seen in sepsis, other pathways seem involved. Myocardial dysfunction during VILI may be calcium-independent. Binding of pulmonary Hsp-70, spilled-over from the lungs, to myocardial TLR-2 with subsequent CXCL-1 expression may play a role in the MV-induced aggravation.

Reference List

1. Kuiper JW, Groeneveld AB, Slutsky AS, Plotz FB. Mechanical ventilation and acute renal failure. *Crit Care Med* 2005;33:1408-15.
2. Slutsky AS, Tremblay LN. Multiple system organ failure. Is mechanical ventilation a contributing factor? *Am J Respir Crit Care Med* 1998;157:1721-5.
3. Kuiper JW, Vaschetto R, Della CF, Plotz FB, Groeneveld AB. Bench-to-bedside review: Ventilation-induced renal injury through systemic mediator release—just theory or a causal relationship? *Crit Care* 2011;15:228.
4. Kuiper JW, Plotz FB, Groeneveld AJ, Haitsma JJ, Jothy S, Vaschetto R et al. High tidal volume mechanical ventilation-induced lung injury in rats is greater after acid instillation than after sepsis-induced acute lung injury, but does not increase systemic inflammation: an experimental study. *BMC Anesthesiol* 2011;11:26.
5. Pelosi P, D'Onofrio D, Chiumello D, Paolo S, Chiara G, Capelozzi VL et al. Pulmonary and extrapulmonary acute respiratory distress syndrome are different. *Eur Respir J Suppl* 2003;42:48s-56s.
6. Sadowitz B, Roy S, Gatto LA, Habashi N, Nieman G. Lung injury induced by sepsis: lessons learned from large animal models and future directions for treatment. *Expert Rev Anti Infect Ther* 2011;9:1169-78.
7. Grandel U, Bennemann U, Buerke M, Hattar K, Seeger W, Grimminger F et al. Staphylococcus aureus alpha-toxin and Escherichia coli hemolysin impair cardiac regional perfusion and contractile function by activating myocardial eicosanoid metabolism in isolated rat hearts. *Crit Care Med* 2009;37:2025-32.
8. Smeding L, van der Laarse WJ, van Veelen TA, Lamberts RR, Niessen HWM, Kneyber MCJ et al. Early myocardial dysfunction is not caused by mitochondrial abnormalities in a rat model of peritonitis. *J Surg Res* 2011; in press.
9. Tavernier B, Mebazaa A, Mateo P, Sys S, Ventura-Clapier R, Veksler V. Phosphorylation-dependent alteration in myofilament Ca²⁺ sensitivity but normal mitochondrial function in septic heart. *Am J Respir Crit Care Med* 2001;163:362-7.
10. Takeuchi K, del Nido PJ, Ibrahim AE, Poutias DN, Glynn P, Cao-Danh H et al. Increased myocardial calcium cycling and reduced myofilament calcium sensitivity in early endotoxemia. *Surgery* 1999;126:231-8.
11. Manny J, Grindlinger G, Mathe AA, Hechtman HB. Positive end-expiratory pressure, lung stretch, and decreased myocardial contractility. *Surgery* 1978;84:127-33.
12. Nin N, Penuelas O, de Paula M, Lorente JA, Fernandez-Segoviano P, Esteban A. Ventilation-induced lung injury in rats is associated with organ injury and systemic inflammation that is attenuated by dexamethasone. *Crit Care Med* 2006;34:1093-8.
13. Brander L, Sinderby C, Lecomte F, Leong-Poi H, Bell D, Beck J et al. Neurally adjusted ventilatory assist decreases ventilator-induced lung injury and non-pulmonary organ dysfunction in rabbits with acute lung injury. *Intensive Care Med* 2009;35:1979-89.
14. Vreugdenhil HA, Haitsma JJ, Jansen KJ, Zijlstra J, Plotz FB, Van Dijk JE et al. Ventilator-induced heat shock protein 70 and cytokine mRNA expression in a model of lipopolysaccharide-induced lung inflammation. *Intensive Care Med* 2003;29:915-22.
15. Hoegl S, Boost KA, Flondor M, Scheiermann P, Muhl H, Pfeilschifter J et al. Short-term exposure to high-pressure ventilation leads to pulmonary biotrauma and systemic inflammation in the rat. *Int J Mol Med* 2008;21:513-9.
16. Mathur S, Walley KR, Wang Y, Indrambarya T, Boyd JH. Extracellular Heat Shock Protein 70 Induces Cardiomyocyte Inflammation and Contractile Dysfunction via TLR2. *Circ J* 2011;75:2445-52.
17. Nijveldt RJ, Prins HA, van Kemenade FJ, Teerlink T, van Lambalgen AA, Boelens PG et al. Low arginine plasma levels do not aggravate renal blood flow after experimental renal ischaemia/reperfusion. *Eur J Vasc Endovasc Surg* 2001;22:232-9.
18. The Acute Respiratory Distress Syndrome Network. Ventilation with lower tidal volumes as compared with traditional tidal volumes for acute lung injury and the acute respiratory distress syndrome. The Acute Respiratory Distress Syndrome Network. *N Engl J Med* 2000;342:1301-8.
19. Lamberts RR, Vaessen RJ, Westerhof N, Stienen GJ. Right ventricular hypertrophy causes impairment of left ventricular diastolic function in the rat. *Basic Res Cardiol* 2007;102:19-27.
20. van der Velden J, Papp Z, Zaremba R, Boontje NM, de Jong JW, Owen VJ et al. Increased Ca²⁺-sensitivity of the contractile apparatus in end-stage human heart failure results from altered phosphorylation of contractile proteins.

Cardiovasc Res 2003;57:37-47.

21. Villar J, Cabrera NE, Casula M, Valladares F, Flores C, Lopez-Aguilar J et al. WNT/beta-catenin signaling is modulated by mechanical ventilation in an experimental model of acute lung injury. *Intensive Care Med* 2011;37:1201-9.
22. Herrera MT, Toledo C, Valladares F, Muros M, Diaz-Flores L, Flores C et al. Positive end-expiratory pressure modulates local and systemic inflammatory responses in a sepsis-induced lung injury model. *Intensive Care Med* 2003;29:1345-53.
23. Dreyfuss D, Saumon G. Ventilator-induced lung injury: lessons from experimental studies. *Am J Respir Crit Care Med* 1998;157:294-323.
24. Smeding L, Lust E, Plotz FB, Groeneveld AB. Clinical implications of heart-lung interactions. *Neth J Med* 2010;68:56-61.
25. Laine GA. Change in (dP/dt)max as an index of myocardial microvascular permeability. *Circ Res* 1987;61:203-8.
26. Verbrugge SJ, Sorm V, van 't Veen A, Mouton JW, Gommers D, Lachmann B. Lung overinflation without positive end-expiratory pressure promotes bacteremia after experimental *Klebsiella pneumoniae* inoculation. *Intensive Care Med* 1998;24:172-7.
27. Nahum A, Hoyt J, Schmitz L, Moody J, Shapiro R, Marini JJ. Effect of mechanical ventilation strategy on dissemination of intratracheally instilled *Escherichia coli* in dogs. *Crit Care Med* 1997;25:1733-43.
28. Mitchell JH, Wildenthal K, Johnson RL, Jr. The effects of acid-base disturbances on cardiovascular and pulmonary function. *Kidney Int* 1972;1:375-89.
29. Villar J, Blanco J, Zhang H, Slutsky AS. Ventilator-induced lung injury and sepsis: two sides of the same coin? *Minerva Anesthesiol* 2011;77:647-53.
30. Mambula SS, Stevenson MA, Ogawa K, Calderwood SK. Mechanisms for Hsp70 secretion: crossing membranes without a leader. *Methods* 2007;43:168-75.
31. Kido T, Bai N, Yatera K, Suzuki H, Meredith A, Mukae H et al. Diesel exhaust inhalation induces heat shock protein 70 expression in vivo. *Inhal Toxicol* 2011;23:593-601.
32. Xiang M, Fan J. Pattern recognition receptor-dependent mechanisms of acute lung injury. *Mol Med* 2010;16:69-82.
33. Boyd JH, Mathur S, Wang Y, Bateman RM, Walley KR. Toll-like receptor stimulation in cardiomyocytes decreases contractility and initiates an NF-kappaB dependent inflammatory response. *Cardiovasc Res* 2006;72:384-93.
34. Zagorski J, Gellar MA, Obraztsova M, Kline JA, Watts JA. Inhibition of CINC-1 decreases right ventricular damage caused by experimental pulmonary embolism in rats. *J Immunol* 2007;179:7820-6.