Effect of Ventilatory Support on Abdominal Fluid Balance in a Sepsis Model

MARCO LATTUADA
Abstract


In patients affected by acute respiratory failure or acute respiratory distress syndrome (ARDS) the leading cause of death is failure of different vital organs other than the lungs, so called multiple organ dysfunction syndrome (MODS). The abdominal organs have a crucial role in the pathogenesis of this syndrome.

There is a lack of knowledge regarding the mechanisms by which mechanical ventilation can affect the abdominal compartment. One hypothesis is that mechanical ventilation can interfere with abdominal fluid balance causing edema and inflammation.

We addressed the question whether different levels of ventilatory support (mechanical ventilation with different levels of positive end-expiratory pressure, PEEP, and spontaneous breathing with or without PEEP) can influence abdominal edema and inflammation in both healthy and endotoxin-exposed animals.

The effect on lymphatic drainage from the abdomen exerted by different degrees of ventilatory support was evaluated (paper I). We demonstrated that endotoxin increases abdominal lymph production, that PEEP and mechanical ventilation increase lymph production but also impede lymphatic drainage; spontaneous breathing improves lymphatic drainage from the abdomen.

By adapting a non-invasive nuclear medicine imaging technique and validating it (paper II), we have been able to evaluate extravascular fluid accumulation (edema formation) in the abdomen over time (paper III) demonstrating that edema increases during endotoxemia, mimicking a sepsis-like condition, and that spontaneous breathing, compared to mechanical ventilation, reduces extravascular fluid. Pro-inflammatory cytokines TNF-α and IL-6 in intestinal biopsies are reduced during spontaneous breathing compared to mechanical ventilation.

Abdominal edema results in increased intra-abdominal pressure (IAP): in paper IV we analyzed the effect of increased intra-abdominal pressure on the respiratory system. Pulmonary shunt fraction increased with high IAP both in healthy and LPS animals, resulting in decreased level of oxygenation. These changes are only partially reversible by reducing IAP.

In conclusion, mechanical ventilation is a life-saving tool but the possible side effect at the extra-pulmonary level should be considered, and the introduction of some degree of spontaneous breathing when clinically possible is a suggested choice.

Keywords: mechanical ventilation, lymph flow, spontaneous breathing, positive end-expiratory pressure, PEEP, abdominal edema, inflammation, intra-abdominal pressure, IAP

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To the Person I Love
List of Papers

The present doctoral thesis is based on the following original papers, which are referred to in the text by their roman numerals.


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Contents

List of Papers ............................................................................................................. v

Contents ....................................................................................................................... vii

Abbreviations ............................................................................................................... 9

Introduction ................................................................................................................... 11
  General aspects ........................................................................................................ 11
  Rationale and Aims of the Thesis ........................................................................... 11
  The Papers .............................................................................................................. 12

Material and Methods ................................................................................................. 13
  Anesthesia ............................................................................................................... 13
  Monitoring ............................................................................................................ 13
  Septic injury .......................................................................................................... 14
  Specific methodologies ........................................................................................... 14
    Lymph flow ........................................................................................................ 14
    Double isotope technique ................................................................................... 15
    Histology .......................................................................................................... 15
    Cytokine measurements ..................................................................................... 16
    V/Q measurement ............................................................................................... 16

Results ......................................................................................................................... 17
  Overview of the results .......................................................................................... 17
  Paper I ................................................................................................................... 17
    Effect of LPS infusion ......................................................................................... 17
    Ventilation and spontaneous breathing ............................................................. 18
    Effect of increased IAP ....................................................................................... 19
  Paper II .................................................................................................................. 19
    Histology ........................................................................................................... 20
  Paper III ............................................................................................................... 21
    Inflammation ....................................................................................................... 21
  Paper IV ............................................................................................................... 23

Discussion .................................................................................................................... 25

Conclusions ................................................................................................................. 29
  Study I ................................................................................................................... 29
Abbreviations

ALI  Acute Lung Injury
APP  Abdominal Perfusion Pressure
ARDS  Acute Respiratory Distress Syndrome
ATM  Atmospheric Pressure
Crs  Compliance of the respiratory system
CO  Cardiac Output
CPAP  Continuous Positive Airway Pressure
CVP  Central Venous Pressure
ELISA  Enzyme Linked Immunosorbent Assay
etCO₂  End-Tidal CO₂ tension
FiO₂  Fraction of Inspired Oxygen
HE  Hematoxylin Eosin
HMW  High Molecular Weight
HR  Heart rate
IAP  Intra-abdominal pressure
In  Indium
IL  Interleukin
LMW  Low Molecular Weight
LPS  Lipopolysaccharide
MAP  Mean Arterial Pressure
MODS  Multiple Organ Dysfunction Syndrome
MV  Mechanical Ventilation
NI  Normalized Index
paCO₂  Partial Pressure of CO₂ in a Arterial blood
paO₂  Partial Pressure of O₂ in a Arterial blood
PAOP  Pulmonary Artery Occlusion Pressure
PAP  Pulmonary Artery Pressure
Paw  Pressure in the airways
PEEP  Positive end-expiratory pressure
PP  Penumoperitoneum
Pv  Pressure in veins
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
</thead>
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<tr>
<td>RBC</td>
<td>Red Blood Cells</td>
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<tr>
<td>ROI</td>
<td>Region Of Interest</td>
</tr>
<tr>
<td>SB</td>
<td>Spontaneous breathing</td>
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<tr>
<td>Tc</td>
<td>Technetium</td>
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<tr>
<td>TD</td>
<td>Thoracic Duct</td>
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<tr>
<td>TNF-α</td>
<td>Tumor Necrosis Factor-alfa</td>
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<tr>
<td>V/Q</td>
<td>Ventilation/Perfusion relationship</td>
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<tr>
<td>Vt</td>
<td>Tidal Volume</td>
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Introduction

General aspects
The pathophysiology of acute respiratory failure refers to a diffuse and overwhelming inflammatory reaction of the pulmonary parenchyma, induced by a variety of underlying diseases (sepsis, severe pneumonia, peritonitis, multiple trauma, lung transplant rejection etc.)(1). In patients affected by acute respiratory failure or acute respiratory distress syndrome (ARDS) the leading cause of death is failure of different vital organs other than the lungs, developing a multiple organ dysfunction syndrome (MODS)(2,4). The abdominal organs have a crucial role in the pathogenesis of this syndrome.

During sepsis, the inflammatory reaction and the activation of the cytokines network results in increase in vascular permeability with fluid extravasation and edema (5,10); in the abdomen the increase in free fluid causes ascites. Capillary fluid reabsorption as well as lymphatic drainage counteract extravascular fluid accumulation (11-14).

Ascitic fluid together with other phenomena (such as increase in intestinal gas/fluid volume because of paralytic ileus) leads to an increase in pressure inside the abdominal cavity (intra-abdominal pressure, IAP). Intra-abdominal hypertension can impede microvascular circulation, with tissue hypoperfusion and ischemia, and can push the diaphragm cranially and impede respiratory excursions (15-21).

Positive pressure mechanical ventilation is a lifesaving tool in patients with acute respiratory failure, but can also be responsible for complications at pulmonary and extra-pulmonary levels (22). The mechanisms of these side effects are different, including increase in intra-thoracic pressure, amplification of the inflammatory response and many others.

Rationale and Aims of the Thesis
The general idea is to study if and how different ventilatory management can influence abdominal fluid balance in its different components (extravascular fluid drainage and accumulation, altered permeability because of inflamma-
tion), and on the other hand to evaluate the effects of increased intra-abdominal pressure on the respiratory system.

The Papers

In paper I the role of lymphatics in draining abdominal extravascular fluid was tested in experimental sepsis. We also compared the effect on thoracic duct lymph flow of mechanical ventilation with different levels of PEEP and spontaneous breathing with a continuous positive airway pressure (CPAP) of 5 cm H$_2$O. In addition, the influence of different outflow pressures was studied. The effect of increased intra-abdominal pressure (IAP) on lymph flow was also tested.

Since abdominal edema is common in sepsis and acute respiratory failure, a technique that observes and measures the phenomenon over time may clarify the mechanisms underlying the clinical observations.

In paper II the purpose was to 1) modify a double isotope technique and test the binding efficiency and kinetics of an intravascular and a diffusible marker and 2) to compare the edema as assessed by the isotope technique with microscopy and physiology measurements. To obtain a range of edema formations we studied both healthy and endotoxin-exposed pigs.

In paper III we addressed the question whether different ventilatory strategies affect abdominal edema formation and whether inflammation in abdominal organs can be associated to the different ventilatory strategies. To study this we applied the double isotope gamma camera technique to evaluate edema formation (as defined in paper II) and observed the animals during the induction of septic injury (LPS infusion) and after allocation into groups: high PEEP (15 cmH$_2$O) in mechanical ventilation, spontaneous breathing in CPAP PEEP 5 cmH$_2$O and mechanical ventilation PEEP 5 cmH$_2$O. The inflammatory response in the intestinal tissue was measured (concentration of pro-inflammatory markers TNF-α and IL-6 in intestinal and pulmonary tissue and plasma).

The intra-abdominal fluid accumulation causes an increase in IAP. Thus paper IV was designed to evaluate the effects of artificially increased IAP on the respiratory system (oxygenation, mechanics, ventilation/perfusion mismatch, morphological changes on CT) in both LPS exposed and control animals. Not only increase in IAP was studied, but also abdominal unloading with decrease of intra-abdominal pressure.
Material and Methods

All the studies were approved by the local animal ethics committee. A total of 98 piglets of Swedish country breeds from a local breeder were used (32 in paper I, 24 in paper II, 30 in paper III, 12 in paper IV).

Anesthesia

Anesthesia was induced with i.m. injection of 0.04 mg/kg atropine (NM Pharma AB, Sweden), 6 mg/kg tiletamine-zolazepam (Zoletil, Vibrac Laboratories), and 2.2 mg/kg xylazine chloride (Rompun, Bayer AG). After induction, a ear vein was cannulated and an opioid, 5 μg/kg fentanyl (Fentanyl B. Braun, Germany), was injected. Muscle relaxation was obtained with 0.25 mg/kg pancuronium bromide (Pavulon, Organon Technika, Sweden) when indicated.

Anesthesia was maintained with a continuous infusion of ketamine 30 mg/kg/h (Ketaminol Vet., Veterinaria AG, Switzerland), 0.1 mg/kg/h midazolam (Dormicum, Roche, Switzerland), 4 μg/kg/h fentanyl and 0.25 mg/kg/h pancuronium bromide in glucose 2.5%. After induction of anesthesia, the pigs were mechanically ventilated (V\(_T\)=10ml·kg\(^{-1}\), FIO\(_2\)=0.50 and PEEP=5cmH\(_2\)O, frequency adjusted to normal arterial pCO\(_2\)) during the surgical preparation and the induction of septic injury (see below).

Monitoring

Cardio-circulatory and respiratory data were recorded continuously.

For cardiovascular monitoring and blood sampling, a branch of the femoral artery (a. Saphena) was cannulated. A triple lumen thermistor-tipped balloon catheter (Swan-Ganz, 7 Fr) was introduced into the pulmonary artery via the right external jugular vein, CO was measured by thermodilution. Through the same access a central venous catheter was inserted.

A tracheotomy was performed and a cuffed tracheal tube (inner diameter, 7 mm) was inserted. The animals were ventilated using Maquet Servo i ventilator (Maquet, Solna, Sweden).

During surgery animals were ventilated in volume-controlled mode, respiratory rate 20-22 breaths/minute, tidal volume (Vt) 10-12 ml/kg, inspira-
tion: expiration ratio of 1:2, positive end-expiratory pressure of 5 cmH₂O, inspired fraction of O₂ 0.5. Minute ventilation was adjusted to normocapnia by changes of frequency or tidal volume. End tidal CO₂ tension was continuously measured.

Septic injury
Sepsis was induced by continuous infusion of endotoxin.

After baseline measurements (30 minutes after the end of surgical manipulations), a continuous intravenous infusion of endotoxin (lipopolysaccharide, LPS, *Escherichia Coli* serotype 0111:B4, diluted in saline) was instituted at the rate of 15 μg/kg/h for 150 minutes. During the remaining part of the experiment endotoxin infusion was continued at 5 μg/kg/h.

Specific methodologies
Specific methodologies have been used and details are given in the single publications. Here is given an overview of the different methodologies.

Lymph flow
Lymph flow was continuously measured by an ultrasonic flow probe around the thoracic duct (TD). The main lymphatic structure that drains lymph from the abdomen to the central circulation was reached at the diaphragm level before its entrance into the thorax.

![Figure 1. Details on the flow probe (right) and actual dimensions (left)](image)
In phase 2 in paper I, the TD was cannulated with an external shunt, collecting lymph in a measuring beaker. The procedure allowed to drain lymph against different outflow pressures.

Double isotope technique

A double-isotope technique was applied: red blood cells (RBC) were used as an intravascular marker and labeled with Technetium 99m ($^{99m}$Tc); transferrin was used as a diffusible marker that mixes with both intravascular and extravascular spaces. This marker was labeled with Indium 111 ($^{111}$In). Images were acquired on a dual-head gamma camera (Infinia™, GE Healthcare) equipped with all-purpose medium-energy collimators. Acquisition was started 20 minutes after injection and planar images were dynamically acquired for 4 hours at a frame rate of 1 frame/15 minutes, measuring the energy emission for the two isotopes simultaneously.

Abdominal edema formation in a selected region of interest (ROI) was assessed by calculating a normalized index (NI):

\[
\text{NI} (t_i) = \frac{\text{In}_{\text{ROI}}(t_i)}{\text{In}_{\text{ROI}}(t_0)} \times \frac{\text{Tc}_{\text{ROI}}(t_i)}{\text{Tc}_{\text{ROI}}(t_0)} \times \frac{\text{In}_{\text{bl}}(t_i)}{\text{In}_{\text{bl}}(t_0)} \times \frac{\text{Tc}_{\text{bl}}(t_i)}{\text{Tc}_{\text{bl}}(t_0)}
\]

where \(\text{In}_{\text{ROI}}\) = ROI transferrin count, \(\text{In}_{\text{bl}}\) = transferrin count in the blood, \(\text{Tc}_{\text{ROI}}\) = RBC count in the ROI, \(\text{Tc}_{\text{bl}}\) = RBC count in the blood, \(t_0\) = time of labeling, \(t_i\) = time of measurement.

Quality test: in four animals, ex-vivo testing of efficiency and stability of labelling was performed. In order to evaluate the efficiency of labeling two 2-ml samples were obtained. One was centrifuged to separate RBC and plasma, in the second sample measurements were made in whole blood. The efficiency of RBC labeling was tested by calculating the ratio of $^{99m}$Tc activity in RBC / whole blood. The efficiency of transferrin labeling was tested by calculating the ratio of $^{111}$In activity in plasma / whole blood. Stability At different time points, plasma proteins were separated using gel-separating columns with a cut-off size of 5 kDa. Fractions of proteins with different molecular weight were tested for $^{111}$In activity.

Histology

In order to test whether NI and objective anatomical changes could be related, considering microscopy as gold standard, the width of intestinal villi and lymphatic vessel diameter in the villi were analysed in 8 animals (4 healthy and 4 LPS exposed animals).

A small (3-5 cm) laparotomy was performed along the right subcostal line under sterile condition. A ribbon marker was placed around a jejunal portion
in order to access the intestine with minimal tissue trauma. Four different intestinal biopsies were taken over time during the protocol.

Tissues were treated according to standard histological methods (standard HE staining) and analyzed for diameter of the lymphatic space along the villus and diameter of the villus itself at the same level (from basal membrane to the opposite basal membrane).

Cytokine measurements
At the end of the protocol, tissue biopsies from intestine, liver and lung were obtained, as well as plasma samples. Pro-inflammatory cytokines TNF-α and IL-6 concentration in tissue sample and plasma were measured by the ELISA technique.

V/Q measurement
Briefly, a mixture of 6 inert gases (sulfur hexafluoride, ethane, cyclopropane, enflurane, diethylether and acetone) were dissolved in Ringer Acetate and infused into a peripheral vein for at least 40 min. At steady state arterial and mixed venous blood samples were obtained and expired gas was collected using a pre-warmed chamber. Inert gas concentrations were measured by gas chromatography (HP 5890; Hewlett-Packard, Waltham, MA) and the blood-gas partition coefficients computed. The ratio between arterial/mixed venous concentration (retention) and mixed expired/mixed venous concentration (excretion) were used to obtain retention-solubility and excretion-solubility correlations. Using a mathematical analysis these correlations were transformed into a 50-compartment distribution model and the ventilation/perfusion relationship estimated.
Results

Overview of the results
In the present thesis we have tested the effect of mechanical ventilation with different levels of PEEP and spontaneous breathing on abdominal fluid balance by evaluating drainage of fluid through the lymphatic pathway, the net effect of extravasation of fluid in the extravascular space with the double isotope technique and finally how the respiratory system is impaired when abdominal edema causes increased IAP.

Thus, mechanical ventilation was associated with an increased level of extra-vascular fluid in the abdomen (ascites) and intestinal swelling (dilated villi) compared to spontaneous breathing (SB). Moreover, the edema was accompanied by an increased inflammatory reaction. Finally, the increased abdominal pressure significantly impaired the respiratory function in both healthy and LPS exposed animals and the abdominal unloading did not restore respiratory function back to pre-loading in the endotoxin-exposed animals during the follow-up period of 30 minutes.

Paper I

Effect of LPS infusion
LPS infusion induced a significant increase in lymph flow from the abdominal cavity. Compared to the intact thoracic duct (study I), lymph flow was lower when the TD was cannulated but a similar increase during the septic injury was observed. When outflow pressure was set at zero (atmospheric pressure), lymph flow was higher compared to an outflow pressure corresponding to central veins (CVP) (figure 2, study 2).
When lymph drainage was drained against zero pressure (ATM) flow was higher compared to the measurement with an outflow pressure equal to CVP (Fig. 2). Spontaneous breathing with CPAP PEEP 5 cmH2O compared to mechanical ventilation resulted in better (higher) lymphatic drainage. Details are presented in Paper I.

Ventilation and spontaneous breathing
Spontaneous breathing with CPAP PEEP 5 cmH2O compared to mechanical ventilation resulted in better (higher) lymphatic drainage from the abdomen as shown in Fig. 2a.
Figure 2a: Percentage change in lymph flow during mechanical ventilation with PEEP 5 or 15 cm H2O and during spontaneous breathing with CPAP 5 cm H2O. Note the higher flow with CPAP.

**Effect of increased IAP**

In healthy animals, lymph flow showed no significant changes over the study period. An increase in IAP tended to lower lymph flow in LPS animals. After deflation, lymph flow increased. For further details, see paper I.

**Paper II**

The results from bench studies showed efficient labeling for the intravascular (non-extravasating) marker (ratio $^{99m}$Tc activity RBC / whole blood): > 95% as well as for the diffusible (extravasating) marker (ratio $^{111}$In activity plasma / whole blood): > 98%.

Moreover, consistent data for validating the double isotope technique in the abdominal compartment were obtained. A synthesis of the changes in NI under different study conditions is given in figure 3. The top line, expression in the increase in NI in the septic animals, correlates with the data from histology.
Figure 3. Changes in NI over time. SB spontaneous breathing, MV mechanical ventilation, LPS lipopolysaccharide.

Histology
The analysis of biopsies was performed by a microscopist unaware of the experimental condition and results correlated with the NI data over time. An example is shown in Fig 4.

Figure 4 Correlation between the diameter of intestinal villi and NI. $\bar{D}$ diameter in $\mu$m.
Paper III

The main result was that SB significantly reduced abdominal fluid accumulation compared to mechanical ventilation, with a non-significant effect for PEEP level (although a mean increase was seen with higher PEEP).

![Figure 5](image)

**Figure 5.** Changes over time in NI in the study groups. Details in the text.

Inflammation

Data from ELISA show that TNF-α and IL-6 in liver and intestine were significantly lower in the spontaneously breathing group with a CPAP of 5 cm H2O compared to both MV groups with either PEEP of 5 or 15 cm H2O (fig. 7).
However, inflammatory marker concentrations in the lung and in plasma were similar in the spontaneously breathing and in the mechanically ventilated animals (see Fig 7).
Figure 7. TNF-α and IL-6 in lung tissue and in plasma. Note the similarity in the inflammatory marker concentrations irrespective of ventilatory mode.

Paper IV

LPS-exposed animals and healthy controls were studied. Increase in intra-abdominal pressure was responsible for severe cardiovascular and respiratory system alterations, with a greater impact under septic conditions (see text).

Pulmonary shunt fraction increased in septic with LPS infusion. Both LPS-exposed and control animals suffered a significant increase in shunt fraction when IAP was increased by CO₂ inflation in the abdomen, even though in control animals shunt increase was significant only after 120 minutes.

Shunt increase was reversible in control animals, but in the septic group the shunt observed after deflation was still significantly higher compared to baseline before PP.
In parallel with the shunt increase there was also an impairment in arterial oxygenation during PP.

![Graph showing shunt changes in LPS and Control animals](image)

**Figure 8.** Shunt changes in the LPS (left) and Control animals (right).

A preliminary CT analysis of morphological changes during PP in both healthy and LPS exposed animals show increase or appearance of densities in the lung and they persisted even after abdominal deflation in the LPS-exposed animals during the 30 minutes that the animals were followed after deflation (Fig. 9).

![CT scan images before and after PP](image)

**Figure 9.** Example of CT scan image at the base of the lung in healthy controls before (left) and after (right) PP. Note the increase in atelectasis, mainly in lower right lung region.
Discussion

Induction of septic injury by endotoxin infusion was accompanied by increased abdominal lymph drainage. Moreover, spontaneous breathing, compared with mechanical ventilation, increased lymph flow from the abdomen to the central circulation.

The positive effect of spontaneous breathing in lymphatic return may be explained by a lower intrathoracic pressure. This should cause less impedance for abdominal fluid to enter the thorax. The presence of muscular activity by itself, with abdominal muscle contraction as well as diaphragmatic activity, can promote the entrance of interstitial fluid into the lymphatic capillaries. In fact, periodic compression and expansion of capillary lymphatics (induced by rhythmic tissue deformations) play a key role in promoting the entrance of interstitial fluid into lymph vessels, and a complex system of valves provides unidirectional flow (23).

Cannulation itself seems to interfere with lymph flow (lower flow in study 2, paper I). Interruption to vessel pumping activity and/or higher resistance due to the presence of the cannula are likely mechanisms.

The higher abdominal lymph flow when draining against atmospheric pressure, i.e. into the beaker, instead of draining back to central circulation against the central venous pressure, suggests that more fluid may enter the extravascular space from the capillaries than can be drained via the lymphatic system. The reason is three-fold. Firstly, the systemic capillary pressure is increased by mechanical ventilation because of increased right atrial pressure. This increases the pressure gradient across the capillary wall, promoting extravasation of plasma. Secondly, the increased intrathoracic pressure compresses the thoracic duct, the major lymph vessel that drains the abdomen and brings lymph back to the blood stream via the subclavian veins. This compression reduces abdominal lymph flow. Thirdly, systemic venous pressure is also increased for the same reason as the capillary pressure and this impedes lymph flow back to the systemic circulation. This is illustrated in Figure 10.
Figure 10. Suggested mechanisms for edema formation in the abdomen. 1. Increased capillary leakage because of impeded vascular return (Pv ↑, increased Pressure in abdominal veins) 2. Impeded lymphatic drainage (compression of thoracic duct in the thorax, and increased venous pressure causing an increased impedance for lymph to enter the systemic circulation).

To get an approximate estimation of how large the abdominal lymph flow is and how much it can be reduced by mechanical ventilation porcine experiments may give some information by comparing abdominal lymph flow during spontaneous breathing and mechanical ventilation. Thus, septic injury by endotoxin infusion was accompanied by increased abdominal lymph drainage during mechanical ventilation with a PEEP of 5 cm H2O from 145 to 350 mL/hr. Spontaneous breathing with a continuous positive airway pressure (CPAP) of 5 cm H2O increased abdominal drainage via the thoracic duct by approximately 100 mL/hr. Simple extrapolation to the weight of humans, assuming all other conditions to be similar to those of the pig, suggests that abdominal edema drainage increases by 250 mL/hr by allowing spontaneous breathing (CPAP) compared with mechanical ventilation, or by approximately 6 L per 24 hrs. Although speculative, the calculation suggests that the lymph drainage may play an important role in edema absorption and that mechanical ventilation may interfere with this adsorption pathway. These rough calculations prompted us to continue with our experiments on abdominal edema formation.
Our results also suggest that spontaneous breathing can promote lymphatic drainage from the abdomen. Under septic conditions, when lymph flow is high because of increased extravascular fluid leakage, spontaneous breathing or possibly the combination of spontaneous and mechanical breaths like in APRV (Airway Pressure Release Ventilation) may prevent or reduce extravascular fluid accumulation.

Abdominal organ edema is a serious complication in case of severe sepsis with possible impairment of barrier function of the intestine with bacterial translocation and reduced organ perfusion (25, 26). For this reason we decided to modify a double isotope technique previously used to estimate edema in the lung (27, 29), in order to follow non-invasively and over time transferring extravascular accumulation (NI, indicating edema).

Possible technical drawbacks, e.g. unbound isotopes or unstable labeling, have been excluded by bench experiments (30).

During LPS infusion, NI increased significantly over time compared to healthy animals, in which NI remained almost constant to 1 during 5 hours: these data are consistent with the expected alteration induced by altered permeability.

We tested if NI changes correlate to an “objective “ measurement of tissue structural changes over time, structural changes that we interpret as reflecting edema formation. Intestinal tissue is sensitive to edema (31-33) and we checked if increased size of lymphatic vessels, indicating activation of the draining system for extravascular fluid and proteins, or villi size were reflected by NI changes. The NI was calculated at the same time point as when biopsies were taken and in the same abdominal regions. Biopsies, although carefully obtained, imply some degree of tissue trauma. We tried to balance the need of serial tissue samples from a similar intestinal region with the risk of tissue damage and bleeding (both conditions that would interfere with microscopy and NI) and evaluated 4 time points, and each point was the average of 15 or more microscopy measurements. NI showed a significant positive correlation with the histological finding both regarding lymphatic vessels and villi dimensions (figure 5). To our knowledge, this is the first study that validates dynamic data from the double isotope technique with serial biopsy findings: these results support the use of NI changes in estimating abdominal fluid and protein accumulation and consequently edema.

We could also see an increase in the concentrations of TNF-α and IL-6 in the intestine and liver with mechanical ventilation and much less so with spontaneous breathing and CPAP. Previous studies have shown increased expression of pro-inflammatory cytokines in abdominal organs in animals exposed to injurious mechanical ventilation (34). In the present study we have also seen an inflammatory response during “conventional” mechanical ventilation with no intention to make the ventilation “injurious”. During spontaneous breathing with CPAP of 5 cm H₂O much less inflammation and edema were seen. Moreover, lung and plasma concentrations of the inflam-
Inflammatory markers did not differ between mechanical ventilation and spontaneous breathing, suggesting that the different concentrations in the abdominal organs were not caused by a spread from the lung or other extra-abdominal organs but rather reflect different degrees of tissue synthesis in the intestine and the liver.

The plasma concentrations of the inflammatory markers were higher than in the abdominal organs. However, the animal had been exposed to intravenous endotoxin infusion that must have provoked an inflammatory response in different circulating cells in the immune system. Moreover, quantitative differences in plasma and tissue concentrations cannot be interpreted as similar quantitative differences in inflammatory response.

All these findings may suggest that the concept “protective ventilation” shall have another focus than being protective against the lung. It might equally well be protective against non-pulmonary organs. In view of the more frequent cause of death by abdominal organ failure than lung failure we suggest that the abdomen should have focus in addition to the lung when creating “protective ventilation”.

Consistently with the study from Quintel et al. (35), in paper IV we were able to demonstrate that increase in intra-abdominal pressure affects pulmonary function in the injured lung. We also tested the effect of increased IAP by PP in the healthy lung. In the literature several papers investigate the effect of increased IAP and PP in distal organs (36-40). We consider it interesting that in our paper we were able to demonstrate that changes induced by increase in IAP are only partially reversible with deflation of the abdomen. This suggests compression atelectasis by the abdominal distension and that the atelectasis remains after deflation. It is possible that a recruitment maneuver of the lung, by raising airway and alveolar pressure to 40 cm H2O or more may have reopened the collapsed lung. It should thus be kept in mind after abdominal deflation.
Conclusions

Study I
In our endotoxin model, the cardiorespiratory effects were consistent with findings seen in clinical sepsis. At the pulmonary level, a transient but dramatic increase in pulmonary artery pressure was observed, with a second rise that after 2.5 hours had reached a steady-state pulmonary hypertension. Lymph flow from the abdomen to the thorax was increased approximately 6 times during the induction of the injury, demonstrating that the increased extravascular fluid that accumulates in the abdomen is at least in part drained by the lymphatic system.

Study II
The double isotope technique was validated for the study of abdominal edema.

NI was significantly higher in the mechanically ventilated animals compared to those that were just mechanically ventilated, and unrestricted spontaneous breathing caused less edema in the abdomen.

Study III
Mechanical ventilation, compared to spontaneous breathing, was associated with increased intra-abdominal edema, as estimated by the double isotope technique. Inflammatory markers in intestinal biopsies were more expressed in the mechanically ventilated animals compared to the spontaneously breathing ones, in our sepsis-like model.

Study IV
An increase in intra-abdominal pressure affected the respiratory system both in endotoxin-exposed and in healthy control animals. Significant effects (increased shunt, decrease in PaO₂) were observed in the LPS-exposed animals. The impairment in respiratory function was not fully reversed with deflation in the septic animals, and a tendency to higher shunt was observed also in the healthy animals.
Perspective and Limits

The thesis opens the important issue of lymphatic drainage. Several aspects still need to be clarified such as lymphatic drainage through the diaphragm of abdominal free fluid, the analysis of the lymph itself that drains back to the central circulation and that can possibly affect the lung. As for lung lymphatic, the measurement of lung lymphatic drainage in different experimental settings is interesting but technically challenging.

In the future, nuclear medicine technique can be used to evaluate continuously edema formation in single abdominal organs, possibly in conjunction with density studies with CT. Functional changes in more prolonged experiments is also a possible future perspective.

No therapeutical interventions were performed because the investigation of the effect of ventilatory support *per se* was the main purpose. Other modes of ventilation as well as different drugs can be tested that can be expected to affect abdominal edema such as vasoactive drugs, diuretics, steroids and others.

Different combinations of spontaneous breathing and mechanical ventilation, or levels of PEEP different than zero, 5 or 15 can be considered, as well as the effect of ventilation on the abdomen in a different injury model (e.g. oleic acid injection, lavage).
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine.