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Effects of Spontaneous Breathing during Airway Pressure Release Ventilation on Intestinal Blood Flow in Experimental Lung Injury

Rudolf Hering, M.D., Andreas Viehöfer, M.D.,† Jörg Zinserling, M.Sc.,‡ Hermann Wrigge, M.D.,* Stefan Kreyer,§ Andreas Berg,§ Thomas Minor, M.D.,‖ Christian Putensen, M.D.#*

Background: **In critical illness, the gut is susceptible to hypoperfusion and hypoxia. Positive-pressure ventilation can affect systemic hemodynamics and regional blood flow distribution, with potentially deleterious effects on the intestinal circulation. The authors hypothesized that spontaneous breathing (SB) with airway pressure release ventilation (APRV) provides better systemic and intestinal blood flow than APRV without SB.**

Methods: **Twelve pigs with oleic acid–induced lung injury received APRV with and without SB. When SB was abolished, either the tidal volume or the ventilator rate was increased to maintain pH and arterial carbon dioxide tension constant as compared to APRV with SB. Systemic hemodynamics were determined by double indicator dilution. Blood flow to the intestinal mucosa–submucosa and muscularis–serosa was measured using colored microspheres.**

Results: **Systemic blood flow increased during APRV with SB. During APRV with SB, mucosal–submucosal blood flow ($ml \cdot g^{-1} \cdot min^{-1}$) was 0.39 ± 0.21 in the stomach, 0.76 ± 0.35 in the duodenum, 0.71 ± 0.35 in the jejunum, 0.71 ± 0.59 in the ileum, and 0.63 ± 0.21 in the colon. During APRV without SB and high tidal volumes, it decreased to 0.19 ± 0.03 in the stomach, 0.42 ± 0.21 in the duodenum, 0.37 ± 0.10 in the jejunum, 0.3 ± 0.14 in the ileum, and 0.41 ± 0.14 in the colon ($P < 0.001$, respectively). During APRV without SB and low tidal volumes, the respective mucosal–submucosal blood flows decreased to 0.24 ± 0.10 ($P < 0.01$), 0.54 ± 0.21 ($P < 0.05$), 0.48 ± 0.17 ($P < 0.01$), 0.43 ± 0.21 ($P < 0.01$), and 0.50 ± 0.17 ($P < 0.001$) as compared to APRV with SB. Muscularis–serosal perfusion decreased during full ventilatory support with high tidal volumes in comparison with APRV with SB.**

Conclusion: **Maintaining SB during APRV was associated with better systemic and intestinal blood flows. Improvements were more pronounced in the mucosal–submucosal layer.**

THE main cause of increased mortality in critically ill patients with acute lung injury is the deterioration of other organ systems, culminating in multiple organ failure.[1] Mechanical ventilation has been suggested to contribute to the development of multiple organ failure by potentiating adverse effects of underlying critical illness on splanchnic hemodynamics.[2,3] Positive-pressure ventilation has been shown to decrease systemic blood flow[4–6] and thereby may deteriorate intestinal perfusion and oxygen supply.[5,7–10] Because inadequate intestinal perfusion is associated with a poor outcome in critically ill patients,[11] it would be of advantage to apply ventilatory strategies that improve gas exchange while having little impact on splanchnic perfusion.

In patients with acute lung injury, partial ventilatory support is increasingly used even in the early phase of the disease.[12–14] Spontaneous breathing with airway pressure release ventilation (APRV), which provides mechanical assistance by time-cycled switching between two levels of continuous positive airway pressure,[15] has been shown to improve gas exchange and systemic blood flow.[16,17] In patients with acute lung injury, renal perfusion has been observed to increase in the presence of spontaneous breathing during APRV.[18] However, it is not known whether spontaneous breathing with APRV is associated with improved intestinal perfusion.

We hypothesized that spontaneous breathing during APRV would improve intestinal perfusion. To test this hypothesis, we determined the intestinal blood flow with the colored microsphere technique during APRV with and without spontaneous breathing in pigs with oleic acid–induced lung injury. Because changes in acid–base homeostasis may significantly alter systemic and regional hemodynamics, arterial carbon dioxide tension ($Paco_2$) was kept constant throughout the study by either increasing tidal volume (V_T) or ventilator rate during full ventilatory support.

Materials and Methods

Instrumentation

The study was conducted in accordance with the *Principles of Laboratory Animal Care*[19] and was approved by the Laboratory Animal Care and Use Committee of the District of Cologne, Germany. Twelve pigs, mixed German country breed, weighing 10–19 kg (15.2 ± 2.9 kg [mean ± SD]) were fasted for 24 h while having free access to water. Before instrumentation, the animals were premedicated with intramuscular ketamine (10 mg/kg), xylazine hydrochloride (2 mg/kg), and glycopyrronium bromide (15 μg/kg) and placed supine on a heating pad to maintain core temperature at 38°C. Anesthesia was induced with intravenous sodium pentobarbital (10 mg/kg) and maintained with sodium pentobarbital ($2 mg \cdot kg^{-1} \cdot h^{-1}$) and ketamine ($2 mg \cdot kg^{-1} \cdot h^{-1}$). The dosage of anesthetics was not changed until the end of the study, when the animals were killed using

* Staff Anesthesiologist, † Resident in Anesthesiology, ‡ Research Associate, § Graduate Medical Student, ‖ Professor, Department of Experimental Surgery, # Professor, Department of Anesthesiology and Intensive Care Medicine, University of Bonn.

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Address reprint requests to Dr. Hering: Department of Anesthesiology and Intensive Care Medicine, University of Bonn, Sigmund-Freud-Strasse 25, D-53105 Bonn, Germany. Address electronic mail to: hering@uni-bonn.de.

an overdose of sodium pentobarbital and potassium chloride. To ensure adequate hydration, 500 ml lactated Ringer's solution was infused rapidly, followed by an infusion rate of 5 ml · kg^{-1} · h^{-1} throughout the study. Animals were tracheotomized, intubated with a 7.5-mm-ID cuffed endotracheal tube (Mallinkrodt, Argyle, NY), and breathed room air spontaneously at ambient airway pressure (P_{aw}) throughout instrumentation.

A 4-French thermistor-tipped pulmonary artery catheter (AI-07044; Arrow International, Inc., Reading, PA) was inserted through the right jugular vein. The left carotid artery was cannulated, and a 4-French pigtail catheter (Duct-Occlud 147420; pfm, Germany) was inserted under pressure control into the left ventricle for injection of microspheres. A 7-French three-lumen catheter (OW-14703-E; Arrow International, Inc.) was placed in the abdominal aorta *via* the right femoral artery for pressure monitoring and blood sampling, and a 4-French thermistor-tipped fiberoptic catheter (Pulsiocath PV2024 l; Pulsion Medical Systems, Munich, Germany) was advanced through the left femoral artery into the descending thoracic aorta. A balloon catheter (International Medical, Zutphen, The Netherlands) was placed in the juxtacardiac portion of the esophagus. At the end of each experiment, the correct position of all catheters was verified by autopsy.

Ventilatory Measurements

Gas flow was measured at the proximal end of the endotracheal tube with a heated pneumotachograph (No. 2; Fleisch, Lausanne, Switzerland) connected to a differential pressure transducer (Huba Control, Würenlos, Switzerland). V_T and minute ventilation were derived from the integrated gas flow signal. V_T was indexed for body weight. Airway pressure was measured at the same position with another differential gas-pressure transducer (SMT, Munich, Germany). To determine esophageal pressure, the esophageal balloon catheter was connected to a differential pressure transducer (SMT), and its position was verified by an occlusion test.[20] Data were sampled *via* an analog–digital converter (DT 2801-a; Data Translation, Marlboro, MA) at sampling intervals of 3 s, processed, and stored on an IBM-compatible personal computer for off-line analysis. The software for data acquisition and evaluation was programmed using a commercially available software tool (Asyst® 4.0; Keithley Asyst, Taunton, MA).

Cardiovascular Measurements

Heart rate was obtained from the electrocardiogram. Mean arterial blood pressure, mean pulmonary artery pressure, and pulmonary artery occlusion pressure were transduced (Combitrans®; Braun AG, Melsungen, Germany), recorded (CS/3; Datex, Helsinki, Finland), and stored on a personal computer at sampling intervals of 5 s. A horizontal plane through the midpoint of the dorsoventral thoracic diameter was taken as zero reference point for pressure measurements. Cardiac output (CO) and intrathoracic blood volume were determined by the transpulmonary double-indicator dilution technique using indocyanine green (Becton Dickinson, Cockeysville, MD) dissolved in iced 5% glucose solution as a double indicator as described previously (COLD-Z-021; Pulsion Medical Systems, Munich, Germany).[21,22] Three measurements performed at random moments during the ventilatory cycle were averaged.[23] Standard formulae were used to calculate stroke volume, pulmonary vascular resistance, and oxygen delivery. Values were indexed for body weight where appropriate.

Blood Gas Analysis

Arterial blood gases and pH were determined immediately after sampling in duplicate with standard blood gas electrodes (ABL 510; Radiometer, Copenhagen, Denmark). In each sample, hemoglobin and oxygen saturation were analyzed using spectrophotometry (OSM 3; Radiometer).

Tissue Blood Flow Measurements

The microsphere technique was used to determine tissue blood flow. In principle, small particles are uniformly mixed with blood after injection into the left ventricle and allowed to circulate freely until they impact in a vessel that is smaller in diameter than the particles. In such a system, the number of particles impacted in a given tissue is proportional to the volume of particle-containing blood perfusing that tissue. Measuring blood flow at different time points in a single animal is possible when using uniquely labeled microspheres at these time points. If the number of particles in the tissue sample is determined and adequate blood flow reference is established, tissue blood flow can be derived. In this study, colored microspheres (Dye Trak®; Triton Technology, San Diego, CA) were used to determine tissue blood flow.[24,25] The polystyrene microspheres are coated with a single colored dye and are 15 ± 0.3 μm in diameter. Red, yellow, blue, violet, or white microspheres were used. Depending on the different absorbance characteristics of each color, 6 to 15 million microspheres suspended in 2–5 ml saline, 0.9%, containing 0.02% Tween 80 were used for each blood flow measurement. Before injection into the left ventricle, the suspension of microspheres was thoroughly sonicated and vortexed. The sequence of the color of microspheres was randomly assigned, and microspheres were injected slowly and continuously so that systemic hemodynamics remained unchanged during injection at all time points. After injection, the catheter was flushed with 10 ml saline. Starting 10 s before the injection of microspheres and continuing for 120 s after the injection was completed, two reference blood samples were withdrawn simultaneously from different lumina of the aortic

catheter at a rate of 5 ml/min with a precision pump (AH 55–2226; Harvard Apparatus GmbH, March-Hugstetten, Germany). At the end of the experiments, animals were killed using sodium pentobarbital and potassium chloride, and the intestines (stomach, duodenum, 50 cm of the proximal jejunum, 50 cm of the distal ileum, 50 cm of the mid colon) and adrenal glands were removed. The tissues were carefully dissected free of adherent fat or connective tissue, and the intestines were opened and rinsed. Intestines were separated by blunt dissection into two layers: the mucosa plus submucosa and the muscularis plus serosa. The validity of the separation was verified histologically by an independent pathologist. The tissues were cut into small samples and weighed (BP 310 S; Sartorius, Germany). Sample weights ranged from approximately 1–2.5 mg. Samples were taken as follows: both adrenal glands, stomach (six pieces of mucosa–submucosa and muscularis–serosa), duodenum (four pieces of mucosa–submucosa and two pieces of muscularis–serosa), jejunum, ileum, and colon (five pieces of mucosa–submucosa and muscularis–serosa). The trapped colored microspheres in each tissue and reference blood samples were quantified by their dye content using spectrophotometry. After digestion of the tissue and blood samples with 4 M potassium hydroxide for at least 24 h at 70°C, the microspheres were harvested on a polyester filter (Nucleopore, pore size 8 μm; Costar, Bodenheim, Germany). The microspheres were washed with 2% Tween 80 and then with ethanol. The dye was recovered from the microspheres by adding 200 μl dimethylformamide. Then, the dye solution was separated from the microspheres by centrifugation at 3,000*g* for 10 min. Because each animal received three differently labeled microspheres, *i.e.*, one unique dye per tested ventilatory mode, the mixed dye solutions contained three different dyes. Spectrophotometric analysis of mixed dye solutions was performed using a spectrophotometer (DU64; Beckmann, Düsseldorf, Germany; wave length range 300–820 nm with 1 nm optical band width). The complex spectra were transferred to a personal computer using the Data-Leader Software (Beckmann), and the composite spectrum of each dye solution was resolved into spectra of the three single constituents using the Dye-Trak® matrix inversion software package (Triton Technologies, Inc.). From the spectrophotometric data of the tissue and blood samples, indicating the absorbance values at the respective wavelengths of the colors being used, and the tissue weight and the reference blood flow, tissue blood flow was calculated using the following equation:[24]

$$\text{tissue blood flow } (\text{mL} \cdot \text{g}^{-1} \cdot \text{min}^{-1}) = A_S \cdot V_{ref} \cdot A_{ref}^{-1} \cdot W_s^{-1}$$

where A_S is the absorbance of the tissue sample, V_{ref} is the reference blood flow, A_{ref} is the mean absorbance of both reference blood samples, and W_s is the weight of the tissue sample. For each organ, the respective median blood flow of all samples was calculated.

Experimental Protocol

After instrumentation, lung injury was induced by intravenous injection of 0.1 ml/kg purified oleic acid over 30 min. An additional 0.2 ml was administered every 30 min until arterial oxygen tension (Pa_{O_2}) was below 50 mmHg during breathing of room air at ambient P_{aw}. Then, the fraction of inspired oxygen was set at 35% and was not changed thereafter. The acute lung injury was allowed to stabilize for 90 min. Pressure-limited ventilatory support was then provided with a demand valve system of a standard ventilator (Evita; Dräger Inc., Lübeck, Germany). The low-pressure level was set at 5 cm H_2O, and the high-pressure level was adjusted to the value corresponding to a V_T of approximately 6 ml/kg. The inspiratory-to-expiratory time ratio was set to 1:1, and the ventilator rate was adjusted to maintain Pa_{CO_2} between 45 and 65 mmHg and to ensure a pH greater than 7.30 during transient neuromuscular blockade with boli of 1 mg/kg intravenous succinylcholine (APRV without spontaneous breathing at low V_T; fig. 1A). Then, the animals were allowed to breathe spontaneously, and the ventilator rate was reduced to maintain Pa_{CO_2} and pH constant (APRV with spontaneous breathing; fig. 1B). Thereafter, spontaneous breathing was again abolished with transient neuromuscular blockade with boli of 1 mg/kg intravenous succinylcholine while the ventilator rate remained low and the high-pressure level was increased to maintain Pa_{CO_2} and pH constant (APRV without spontaneous breathing at high V_T; fig. 1C). During these prerandomization periods, at least 15 min was provided for equilibration before measurements of Pa_{CO_2} and pH. After ventilator settings were defined, the animals were assigned with sealed envelopes to receive APRV with spontaneous breathing, APRV without spontaneous breathing using low V_T, and APRV without spontaneous breathing using high V_T in random order, and measurements were performed in each mode. To guarantee absence of spontaneous breathing during full ventilatory support neuromuscular blockade was induced with 0.15 mg/kg intravenous cisatracurium. Absence of spontaneous breathing was verified from on-line registration of the esophageal pressure tracings. After the ventilatory modality was changed, 30 min of equilibration was allowed before measurements. Then, ventilatory, gas exchange, and hemodynamic data were recorded during a period of 30 min.

Statistical Analysis

To detect differences in intestinal blood flow between the ventilatory settings with the given two-sided crossover design at a significance level of 5% ($\alpha = 0.05$) with a probability of 80% ($\beta = 0.20$), based on an estimated

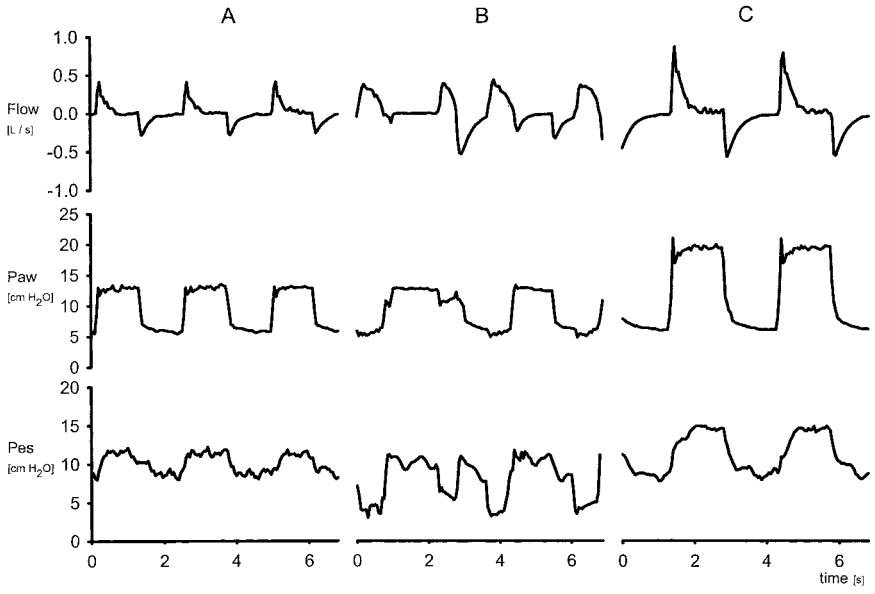

Fig. 1. Original tracings (pig 6) of gas flow, airway pressure (P_{aw}), and esophageal pressure (P_{es}) time courses. (*A*) Airway pressure release ventilation (APRV) without spontaneous breathing using low tidal volume (*B*) APRV with spontaneous breathing. (*C*) APRV without spontaneous breathing using high tidal volume. Note that APRV without spontaneous breathing is identical to time-cycled, pressure-controlled mechanical ventilation. In the absence of spontaneous breathing, either the ventilator rate (*A*) or the upper P_{aw} limit (*C*) was increased to ensure constant arterial carbon dioxide tension and pH as compared to APRV with spontaneous breathing (*B*).

difference of 0.78 of the within-animal SD of intestinal blood flow, the number of animals to be studied was at least 12. Results are expressed as mean ± SD. Data were evaluated for normal distribution with the Shapiro-Wilks W test. To verify adequate mixing of microspheres in the blood circulation and even distribution of blood flow to the various organs after injection into the left ventricle, correlations were calculated between the numbers of microspheres trapped in the two reference blood samples as well as between blood flows to the right and left adrenal glands by using linear regression analysis. Ventilatory, lung mechanic, gas exchange, and systemic hemodynamic data obtained during the different ventilatory modes were compared using one-way analysis of variance. Data of intestinal blood flow were analyzed using two-way analysis of variance with the ventilatory modality as the within-group factor and the mucosa–submucosal and the muscularis–serosal blood flow as the between-groups factor. When a significant F ratio was obtained, differences between the means were isolated by the Newman-Keuls test. Differences were considered to be statistically significant if P was less than 0.05.

Results

During APRV, spontaneous breathing accounted for approximately one half of the total minute ventilation (table 1). To maintain pH and $Paco_2$ constant during APRV without spontaneous breathing, either the high P_{aw} level had to be elevated, resulting in an increase in V_T and mean P_{aw} ($P < 0.001$), or the ventilator rate had to be increased ($P < 0.001$) while mean P_{aw} remained constant (table 1). Mean esophageal pressure was highest when P_{aw} and V_T were increased to compensate for the suppressed spontaneous breathing ($P < 0.001$; table 1). Spontaneous breathing during APRV resulted in the lowest esophageal pressure ($P < 0.01$; table 1).

Spontaneous breathing during APRV resulted in an increase in Pao_2, stroke volume ($P < 0.05$), CO, intratho-

Table 1. Lung Mechanic and Ventilatory Variables

	APRV with Spontaneous Breathing*	APRV without Spontaneous Breathing, High V_T*	APRV without Spontaneous Breathing, Low V_T*
FIO_2, %	35 ± 7	35 ± 7	35 ± 7
P_{low}, cm H_2O	5 ± 0	5 ± 0	5 ± 0
P_{high}, cm H_2O	14 ± 3	22 ± 3§	15 ± 3#
RR, min^{-1}	40 ± 10	18 ± 7§	52 ± 10§#
V_T, ml/kg	7.2 ± 1.4	11.3 ± 3.1§	5.6 ± 1.7#
$\dot{V}_E$, l/min	4.1 ± 0.7	3.5 ± 1.0†	4.6 ± 1.0‖
$\dot{V}_E$ spontaneous breaths, %	54 ± 10	0 ± 0§	0 ± 0§
Mean P_{aw}, cm H_2O	11.3 ± 1.7	15.7 ± 2.1§	12.2 ± 1.4#
Mean P_{es}, cm H_2O	8.7 ± 4.5	10.5 ± 4.5§	9.4 ± 4.5‡#

Values are mean ± SD.

* Tested on a randomized basis. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$ compared with airway pressure release ventilation (APRV) with spontaneous breathing. ‖ $P < 0.05$, # $P < 0.001$ compared with APRV without spontaneous breathing, high tidal volume (V_T).

FIO_2 = fraction of inspired oxygen; P_{aw} = airway pressure; P_{es} = esophageal pressure; P_{high} = high airway pressure level; P_{low} = low airway pressure level; RR = respiratory rate; $\dot{V}_E$ = minute ventilation.

Table 2. Gas Exchange and Systemic Hemodynamic Variables

	APRV with Spontaneous Breathing*	APRV without Spontaneous Breathing, High V_T*	APRV without Spontaneous Breathing, Low V_T*
HR, min^{-1}	128 ± 35	148 ± 31†	144 ± 42
MAP, mmHg	97 ± 14	84 ± 14‡	93 ± 10#
MPAP, mmHg	26 ± 3	26 ± 7	27 ± 3
PAOP, mmHg	11 ± 3	11 ± 3	11 ± 3
ITBV, ml/kg	23 ± 3	19 ± 3§	21 ± 3‡#
CO, $ml \cdot kg^{-1} \cdot min^{-1}$	141 ± 28	110 ± 10§	127 ± 17‡#
SV, $ml \cdot kg^{-1} \cdot beat^{-1}$	1.1 ± 0.28	0.8 ± 0.10§	0.9 ± 0.28†‖
PVR, $mmHg \cdot kg \cdot min \cdot ml^{-1}$	0.11 ± 0.03	0.14 ± 0.03†	0.13 ± 0.03
Hemoglobin, g/dl	9.7 ± 0.7	9.8 ± 0.7	9.9 ± 1.0
Sa_{O_2}, %	98 ± 3	96 ± 3	93 ± 7†
Pa_{O_2}/F_{IO_2}, mmHg	332 ± 100	269 ± 83†	249 ± 83†
$\dot{D}_{O_2}$, $ml \cdot kg^{-1} \cdot min^{-1}$	18.5 ± 4.5	14.1 ± 1.7§	15.6 ± 1.4‡
Pa_{CO_2}, mmHg	56 ± 10	55 ± 10	58 ± 10
pH	7.36 ± 0.07	7.36 ± 0.07	7.35 ± 0.10

Values are mean ± SD.

* Tested on a randomized basis. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$ compared with airway pressure release ventilation (APRV) with spontaneous breathing. ‖ $P < 0.05$, # $P < 0.01$ compared with APRV without spontaneous breathing, high tidal volume (V_T).

CO = cardiac output; $\dot{D}_{O_2}$ = oxygen delivery; F_{IO_2} = fraction of inspired oxygen; HR = heart rate; ITBV = intrathoracic blood volume; MAP = mean arterial pressure; MPAP = mean pulmonary arterial pressure; Pa_{CO_2} = arterial carbon dioxide tension; Pa_{O_2} = arterial oxygen tension; PAOP = pulmonary artery occlusion pressure; Sa_{O_2} = arterial oxygen saturation; SV = stroke volume.

racic blood volume, and oxygen delivery ($P < 0.01$; table 2). In the absence of spontaneous breathing, mean arterial pressure, intrathoracic blood volume, CO ($P < 0.01$), stroke volume ($P < 0.05$), and oxygen delivery were lowest, and heart rate and pulmonary vascular resistance were highest during mechanical ventilation with high V_T (table 2). Mean pulmonary arterial pressure and pulmonary artery occlusion pressure were not different between the tested modalities (table 2).

Adequate mixing of injected microspheres and even distribution of blood flow to the various organs was indicated by highly significant correlations between the numbers of microspheres trapped in the two reference blood samples (5,575 ± 1,652 *vs.* 5,413 ± 1,413 microspheres per sample, $r = 0.91$) and blood flows to the right and left adrenal glands (1.75 ± 0.59 $ml \cdot g^{-1} \cdot min^{-1}$ *vs.* 1.76 ± 0.66 $ml \cdot g^{-1} \cdot min^{-1}$, $r = 0.90$) ($P < 0.0001$).

Transmural distribution of blood flow showed better perfusion of the mucosal–submucosal layer as compared to the muscularis–serosal layer throughout the intestines ($P < 0.01$; table 3). Intestinal blood flow was lowest without spontaneous breathing during APRV at high V_T (table 3). Spontaneous breathing with APRV markedly increased the blood flow to the mucosa–submucosa at all sites of the intestines ($P < 0.05$; table 3). Muscularis–serosal perfusion improved with spontaneous breathing in all intestinal areas except for the gastric muscularis–serosal perfusion when compared to APRV without spontaneous breathing at high V_T ($P < 0.05$; table 3). When compared to APRV without spontaneous breathing at low V_T, the difference in muscularis–serosal per-

Table 3. Intestinal Blood Flow ($mL \cdot g$ wet $tissue^{-1} \cdot min^{-1}$)

	APRV with Spontaneous Breathing*	APRV without Spontaneous Breathing, High V_T*	APRV without Spontaneous Breathing, Low V_T*
Mucosa–submucosa			
Stomach	0.39 ± 0.21	0.19 ± 0.03§	0.24 ± 0.10‡
Duodenum	0.76 ± 0.35	0.42 ± 0.21§	0.54 ± 0.21†
Jejunum	0.71 ± 0.35	0.37 ± 0.10§	0.48 ± 0.17‡
Ileum	0.71 ± 0.59	0.30 ± 0.14§	0.43 ± 0.21‡
Colon	0.63 ± 0.21	0.41 ± 0.14§	0.50 ± 0.17§‖
Muscularis–serosa			
Stomach	0.26 ± 0.48**	0.18 ± 0.45	0.20 ± 0.42
Duodenum	0.37 ± 0.35††	0.13 ± 0.21†**	0.18 ± 0.21††
Jejunum	0.29 ± 0.38††	0.09 ± 0.10†**	0.13 ± 0.17††
Ileum	0.16 ± 0.21††	0.06 ± 0.07†#	0.08 ± 0.07††
Colon	0.07 ± 0.03††	0.03 ± 0.03† ††	0.05 ± 0.03††

Values are mean ± SD.

* Tested on a randomized basis. † $P < 0.05$, ‡ $P < 0.01$, § $P < 0.001$ compared with airway pressure release ventilation (APRV) with spontaneous breathing. ‖ $P < 0.01$ compared with APRV without spontaneous breathing, high tidal volume (V_T). # $P < 0.05$, ** $P < 0.01$, †† $P < 0.001$ compared with mucosa–submucosa of the same intestinal region.

fusion was not significant (table 3). Comparing controlled mechanical ventilation at high or low V_T, there was no difference in intestinal blood flow, with the exception of the colonic mucosal–submucosal perfusion ($P < 0.05$; table 3).

Adrenal perfusion was not different in the presence of spontaneous breathing (2.02 ± 1.28 ml $\cdot$ g^{-1} $\cdot$ min^{-1}) as compared to full ventilatory support applying high (1.6 ± 0.76 ml $\cdot$ g^{-1} $\cdot$ min^{-1}) or low V_T (1.74 ± 0.66 ml $\cdot$ g^{-1} $\cdot$ min^{-1}).

Discussion

The aim of our study was to investigate the effects of spontaneous breathing during APRV on intestinal blood flow in an animal model with lung injury. We found that spontaneous breathing with APRV improved arterial oxygenation, systemic hemodynamics, and regional blood flow to the stomach and the small and large bowels when compared to full ventilatory support at constant $Paco_2$ and pH. Improvement in blood flow with spontaneous breathing was more pronounced in the mucosal–submucosal as compared to the muscularis–serosal layer.

Partial ventilatory support is commonly used not only to separate patients from mechanical ventilation, but also to provide stable ventilatory assistance of a desired degree during ventilatory failure.[12–14] We used APRV that provides a constant degree of ventilatory support by time-cycled switching between two levels of continuous positive airway pressure, allowing spontaneous breathing in any phase of the mechanical ventilator cycle.[15,25,26] When spontaneous breathing is abolished, APRV is not different from conventional pressure-controlled mechanical ventilation.[15,25,26] In our animals, either the respiratory rate or the upper P_{aw} limits were increased in the absence of spontaneous breathing to compensate for the decrease in alveolar ventilation and to maintain $Paco_2$ and pH constant. Therefore, our results should essentially reflect the effect of spontaneous breathing on systemic and intestinal blood flow.

Direct measurement of intestinal perfusion is not possible in critically ill patients. Therefore, we investigated pigs with oleic acid–induced lung injury and used the colored microsphere technique, which is a standard method to determine regional blood flow in experimental animals.[23,24] The theoretical basis of the microsphere technique is analogous to that of the indicator-dilution method, and measuring blood flow with microspheres is limited to distinct time points. A major advantage of this method is its ability to quantitate blood flow to individual tissue regions such as separate layers of the intestinal wall at multiple intestinal locations.[23] We chose microspheres of 15 μm in diameter because shunting of 15-μm microspheres has been found to be negligible, and blood flow measured with microspheres of that size represents nutritive blood flow.[27] Although it has been demonstrated that even serial injections of up to 48 million microspheres have no significant effects on systemic and regional blood flow,[28] we randomized the sequence of the ventilatory support modalities to exclude any confounding effects of serial measurements on regional blood flow. Surgical stress can significantly alter the splanchnic blood flow.[29] However, the measurement of intestinal perfusion with microspheres does not necessitate laparotomy and manipulation of intestinal vessels before measurements. In contrast, surgical implantation of catheters is necessary with other methods such as electromagnetic flow measurements. Even tonometry, which seems to be a feasible method to monitor gastric intramucosal pH noninvasively, would require laparotomy to assess the mucosal energy balance of the bowels. Therefore, to study the effects of different modes of ventilatory support in experimental lung injury being undisturbed by any other intervention, the microsphere technique seemed to be most appropriate. The validity of using microspheres to study regional blood flow distribution is based on adequate mixing of injected microspheres and even distribution to the various parts of the body. This was demonstrated in our study by the close correlation between the numbers of microspheres trapped in the reference blood samples from different sites in the aorta and nearly identical blood flows to both adrenal glands.

Because the mucosal–submucosal and muscularis–serosal layers of the intestinal wall have different metabolic and oxygen requirements,[30] with the mucosa–submucosa representing the layer with the highest demand, we measured blood flow to the different layers separately. Blood flows to both layers were consistent with previous flow measurements using the colored microspheres technique.[23] Because the submucosa has a very small capillary network, it only receives approximately 1.5% of the entire intestinal nutritive blood flow.[31] Therefore, including the submucosal with mucosal blood flow may have minimally overestimated the true mucosal blood flow.

Spontaneous breathing with APRV consistently improved arterial oxygen tension, which is in agreement with previous studies demonstrating a decrease in intrapulmonary shunting by spontaneous breathing with APRV.[13,14,16–18] In addition, spontaneous breathing was associated with an increase in systemic blood flow, which, in combination with the better arterial oxygenation, resulted in improved oxygen delivery to the tissues. Our data indicate that the better CO was not mediated by a change in heart rate but was caused by an increase in stroke volume. We suggest that the improved cardiac preload accounted for the better cardiac performance in the presence of spontaneous breathing. In agreement with previous studies,[17,18] intrathoracic blood volume, which is a reliable surrogate marker of

cardiac preload during mechanical ventilation,[32] was highest in the presence of spontaneous breathing. These data support the concept that spontaneous breathing increases venous return to the heart by periodically lowering intrathoracic pressure.[33,34] In contrast, increase in intrathoracic pressure during controlled mechanical ventilation has been shown to impede venous return and CO.[35] When spontaneous breathing was abolished during our study, mean esophageal pressure as a surrogate parameter of pleural pressure increased, and venous return and CO decreased. In particular, increasing V_T to compensate for the reduction in minute ventilation resulted in a marked increase in pulmonary vascular resistance, reflecting elevated right ventricular outflow impedance. This observation is consistent with previous findings[35,36] that P_{aw} and V_T are major determinants of right-sided heart performance during mechanical ventilation and confirms results of earlier studies demonstrating the impact of large V_T on the impairment in systemic and regional blood flow.[36,37] The hemodynamic changes in our study were unlikely to result from an increase in sympathetic outflow because adrenal blood flow remained unchanged, and heart rate was lowest in the presence of spontaneous breathing.

The intestines are particularly vulnerable to hypoperfusion because the reduction of splanchnic perfusion is more pronounced and out of proportion with the decrease in systemic blood flow whenever the circulation is subjected to low-flow conditions.[7,38] Any reduction of splanchnic blood flow by mechanical ventilation in the critically ill should be prevented because the restoration of splanchnic circulation often remains incomplete, even if resuscitation was effective to restore systemic blood flow.[8,39] In this study, the improvement in systemic blood flow during APRV with spontaneous breathing was associated with an improvement in intestinal blood flow. In accordance with previous studies that documented a reduction in splanchnic blood flow with an increase in the level of positive end-expiratory pressure during mechanical ventilation[7–10] or after switching from spontaneous breathing to continuous positive-pressure ventilation,[5] our findings support the contention that an increase in the level of ventilatory support can deteriorate splanchnic hemodynamics. However, our data do not allow one to distinguish whether intestinal blood flow increased because changes in arterial inflow caused by the improved systemic blood flow alone or, in addition, by changes in mesenteric vascular tone.

Irrespective of the applied ventilatory mode, mucosal–submucosal exceeded muscularis–serosal perfusion, which is in accordance with previous investigations in different mammalian species[30] and reflects the high metabolic activity of the intestinal mucosa. These high substrate requirements, as well as the unique anatomic arrangement of the nutritive vessels, make the mucosal cells at the top of the intestinal villi extremely susceptible to hypoxia: the artery and the corresponding veins run in parallel, allowing a countercurrent exchange of oxygen, resulting in a descending gradient of oxygen from the base to the tip of the villus.[40] It is noteworthy that the decrease in intestinal blood flow observed during full mechanical ventilation with high P_{aw} and V_T was close to or even below the "critical" range where the oxygen demand of intestinal tissues has been observed to become supply dependent in isolated pump-perfused bowel preparations.[41] One might speculate that during mechanical ventilation, intestinal blood flow falls even earlier below this critical range in the presence of specific comorbidity such as hemodynamic shock and/or intraabdominal pathology, being frequently observed in critically ill patients. Therefore, it seems to be of clinical relevance that maintaining spontaneous breathing with APRV was even more effective in improving intestinal mucosal–submucosal perfusion than reducing P_{aw} and V_T and, concomitant with the better oxygenation, essentially improved mucosal oxygen supply throughout the intestine.

Although the metabolic activity of the intestinal muscularis–serosal layer is smaller than that of the mucosal–submucosal layer, poor perfusion of this layer can also affect intestinal integrity.[30] The overall impairment in muscularis–serosal perfusion was less pronounced than in the mucosal–submucosal layer, and the decrease in muscularis–serosal blood flow with increasing P_{aw} and V_T in the absence of spontaneous breathing reflected a typical dose–response relation. Differences in the effectiveness of the mechanisms that control blood flow to the different intestinal layers probably account for the various responses to the change in ventilatory modality. Although myogenic intrinsic control of perfusion has been shown to be dominant in the mucosa–submucosa, the metabolic control mechanism has been shown to be dominant in the muscularis–serosa.[42] Because blood flow and oxygen supply decreased in the absence of spontaneous breathing, accumulation of vasodilator metabolites and the decrease in oxygen tension might have induced vasodilation, resulting in partial restoration of blood flow to the muscularis–serosa.

In conclusion, our results add further information to previous findings that the intestinal circulation can be essentially influenced by the choice of the ventilatory support modality. We demonstrated that partial ventilatory support using spontaneous breathing with APRV improved intestinal blood flow as compared to full ventilatory support. These alterations in intestinal blood flow may have important clinical implications because the splanchnic organs play a key role in the cascade of multiple organ failure,[2,3] which is the main cause of increased mortality in patients with acute lung injury.[1] Therefore, these experimental data should promote further research to elucidate the potential of maintaining

spontaneous breathing with APRV to improve intestinal perfusion in critically ill patients.

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