Impact of bronchial circulation on bronchial exudation following combined burn and smoke inhalation injury in sheep

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Abstract

We previously reported bronchial circulation contributes to pulmonary edema and increases shunt fraction following smoke inhalation, and bronchial blood flow significantly increases in inhalation injury. We hypothesized reduction of bronchial blood flow reduces exudation to the airway and ameliorates lung injury from combined burn and smoke insults (B & S injury).

Method: Merino ewes (n=28) randomly divided into three groups (1: bronchial artery ligated and injured; 2: bronchial artery left intact and injured; 3: bronchial artery ligated but not injured) were subjected to a flame burn and inhalation injury under halothane anesthesia. Parameters were analyzed using Scheffe's post hoc test ($P<0.05$). All Groups were resuscitated with Ringer lactate solution and placed on a ventilator for 48 hours.

Results: Pulmonary gas exchange (PaO_2/FiO_2) improved in Group 1. Further, obstruction score, an index of airway cast formation, significantly changed in Group 2 compared to Group 3 (bronchioli: Groups 2 vs. 1 vs. 3; 10.693 ± 2.848 vs. 5.294 ± 1.14 vs. 2.883 ± 0.512: mean ± SE).

Conclusion: Bronchial circulation plays a significant role in lung injury after B & S injury, and reduction of bronchial blood flow by bronchial artery ligation reduces bronchial exudation, resulting in improved gas exchange.

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Introduction

In the case of severe trauma such as burn injury, it is generally recognized that multiple organ failure (MOF) can be a fatal consequence. Acute respiratory distress syndrome (ARDS) is the first pathology in this sequelae, followed by renal and cardiac and hepatic dysfunction, and finally gastrointestinal dysfunction [1]. Upon return from the injured organ, whole blood containing cellular debris and toxic mediators must eventually pass through the lung and be exposed to pulmonary microcirculation [2]. Especially in instances of severe inhalation injury, prophylaxis against ARDS is essential.

The blood flow of the lower trachea is supplied by the systemic circulation of the bronchial arteries. Immediately following inhalation injury, there is a 20-fold increase in bronchial blood flow, resulting in lung edema formation [3]. Pulmonary edema may be produced by increased filtration from either the pulmonary or bronchial circulation.

Exudation of plasma into the airway contributes to the formation of the obstructive cast and results in increased airway pressure. The formation of an obstructive airway cast following smoke inhalation injury may cause atelectasis, pneumonia, and barotraumas.

The acute phase of ALI is characterized by the influx of protein-rich edema fluid into the air spaces as a consequence of increased permeability of the alveolar

capillary barrier. Sakurai et al. [4] showed that the reduction of bronchial circulation ameliorates the increased pulmonary transvascular fluid flux stemming from the ablation of the bronchial artery in the case of smoke inhalation injury over a 24-h period. Therefore, the purpose of the present study is to use our well established ovine model of combined burn and smoke inhalation injury (B & S injury) to determine whether the reduction of bronchial artery blood flow can effectively ameliorate lung edema, bronchial exudation, and lung damage over a 48-h period.

Materials and Methods

Animals

Twenty-eight adult, female sheep were cared for in the Investigative Intensive Care Unit at the University of Texas Medical Branch, Galveston, TX. The experimental procedure was approved by the Animal Care and Use Committee of the University of Texas Medical Branch. The National Institutes of Health and American Physiological Society guidelines for animal care were strictly followed.

Surgical preparation

Sheep (33 ± 5 kg) were surgically prepared as described previously [5, 6]. A Swan-Ganz thermal dilution catheter was inserted through the right external jugular vein (model 93A-1317-F, Edwards Critical Care Division; Irvine, CA) to measure core body temperature, evaluate blood gas levels (PvO2), and for fluid resuscitation purposes from introducer of Swan-Ganz catheter to Jugular vein. An arterial catheter was inserted into the right femoral artery (16 Gauge, 24 inch. Intracath, Becton Dickinson; Sandy, UT) for the measurement of arterial blood gases. The caudal mediastinal lymph node was cannulated (silastic medial grade tubing, 0.025 in. ID, 0.047 in. OD, Dow Corning; Midland, MI) according to a modification of the technique described by Staub et al. [7]. Vascular occluders (In Vivo Metric System, Healdsburg, CA) were placed at the left pulmonary artery as it entered the left lung as previously

described by Isago et al. [8]. A Silastic catheter was positioned in the left atrium during the procedure to inject the colored microsphere and measure left atrial pressure directory. The contribution to the lymph node was removed by ligation of the tail of the caudal mediastinal lymph node and cauterization of the systemic diaphragmatic lymph vessels [9]. After the operation, 0.5mg of buprenorphine was given by intramuscular injection and also 48hs post-surgery. The sheep were given 5–7 days to recover from the operative procedure and provided food and water *ad libitum*.

Animal grouping

The animals were randomized into three groups as follows: 1) Bronchial artery ligation group (Injury + ligation group: n=10); the left thorax was reopened and bronchoesophageal artery was then exposed, and the bronchial artery was ligated with 5-0 silk suture. 2) Non-ligation group (Injury + no ligation group: n=12); the bronchial artery was surgically exposed but left intact without ligation. Both groups 1 and 2 were given a B & S injury. 3) Sham group (No injury + ligation group: n=6); the bronchial artery was ligated but B & S injury was not administered.

Ablation of bronchial artery

After a 5–7 day recovery period, the animals were endotracheally intubated and ventilated during surgery, which was performed under halothane anesthesia. The left thorax was reopened through the fifth intercostal space, and the lungs were

retracted, exposing the dorsal anatomy. During this procedure, a pleural adhesiotomy was performed. Thereafter, the bronchoesophageal artery was exposed and the bronchial artery ligated with 5-0 silk suture (Injury + ligation and no injury + ligation groups). In the injury + no ligation group, the bronchoesophageal and bronchial arteries were exposed but left intact.

To substantiate the changes in bronchial blood flow, colored microspheres (Interactive Medical Technologies, West Los Angeles, CA) were injected immediately before and after the second operation (bronchial artery ligation operation), 6, 12, 24, and 48 h after the B & S injury [3]. Approximately 12×10^6 fluorescent colored microspheres (15.0±0.1 μm) were injected into the left atrium by catheter just after the left pulmonary artery occlusion using occluders set at the left pulmonary artery. To calibrate microsphere numbers per blood flow, blood was withdrawn from the aorta with a Harvard pump (Harvard Apparatus model 55-1143, South Natick, MA) at a rate of 10 mL/min; the withdrawal was started before microsphere injection and continued for 2 min. Tissue samples of left bronchi, 2–4 mm (proximal bronchi) and 1–2 mm (distal bronchi) in diameter, were obtained post-mortem and used to quantify the bronchial blood flow to the intraparenchymal regions before and after the second operations (0h) and 6, 12, 24, and 48 h after injury.

Burn and smoke inhalation injury (B & S injury)

The protocol has been described previously [5, 6]. 24h after the second operation, this procedure was performed. We gave buprenorphine 0.3mg by intramuscular injection just before the injury. Briefly, after induction of anesthesia with 10 mg/kg ketamine (Ketalar; Parke-Davis; Morris Plains, NJ), a tracheotomy was performed, and a Shiley tracheotomy tube (10-mm diameter, Shiley; Irvine, CA) was inserted and also foley catheter was inserted to collect the urine. Anesthesia was maintained with halothane, and B & S injury was given to the ligation and control groups. Using a Bunsen burner, a 20% total body surface area, third-degree flame burn (The flame was applied till the shrinking of skin stops and the redness disappears) was made on one flank. For this procedure, we covered the pulmonary artery occluders, LA catheter and the lymph collection line by steel plate to avoid the thermal damage to these lines. Thereafter, inhalation injury was induced while the sheep were in the prone position as described previously [5, 6]. A modified bee smoker was filled with 50 g of burning cotton toweling and connected to the tracheostomy tube via a modified endothoracheal tube containing an indwelling thermistor from a Swan-Ganz™ catheter. During the insufflation procedure, the temperature of the smoke did not exceed 40°C to prevent thermal injury to the airway. The sheep were insufflated with a total of 48 breaths of cotton smoke. After smoke insufflations, another 20% total body surface area (for a total of 40% body surface area burned) third-degree burn was made on the contra-lateral flank.

Resuscitation protocol

The protocol has been described previously [5, 6]. Immediately after the B & S injury, anesthesia was discontinued and the animals were allowed to awaken. They were mechanically ventilated with a Servo ventilator (model 900C, Siemens-Elena; Solna, Sweden) throughout the following 48-h experimental period. Ventilation was performed with a positive end-expiratory pressure (PEEP) of 5 cm H_2O and a tidal volume of 15 mL/kg. The respiratory rate was set to maintain normo-capnea. For the first 3 h post-injury, all animals received an inspired oxygen concentration (FiO_2) of 100% to expedite the removal of CO; thereafter, FiO_2 was adjusted to maintain the arterial oxygen saturation at >90%. These respiratory settings allowed rapid carboxyhemoglobin clearance after smoke inhalation.

During the experiment, fluid resuscitation was performed with Ringer's lactate solution using the following the Parkland formula: 4 mL/% burned surface area/kg body wt for the next 24 h, and 2 mL/% burned surface area/kg body wt per day for the following 48 h; an equivalent amount of fluid was administered to the sham animals [5, 6]. During this experimental period, the animals were freely allowed access to food, but not to water, to allow for accurate determination of fluid balance.

Measured variables

Measured physiological variables were not considered valid until the animals were fully awake and standing, which usually occurred within 1 h post injury. Arterial

blood was measured with a blood gas analyzer (model IL1600; Instrumentation Laboratory; Lexington, MA). The data were corrected for core body temperature. Lung lymph flow was measured with a graduated test tube and stopwatch. Lymph and blood samples were collected in EDTA tubes, and the total protein concentration in plasma (CP) and lymph (CL) were measured with a refractometer (National Instrument; Baltimore, MD). Thereafter, pulmonary microvascular permeability index (PI) was calculated by the following equation: PI=QL× (CL/CP), where QL is lung lymph flow (mL/h) [5].

Forty-eight hours after insult, when all measurements were completed, the animals were anesthetized with ketamine and humanely euthanized by administration of 60 mL of saturated potassium chloride solution i.v. Immediately after this procedure, the right whole lower lobe of the lung was harvested for pathological examination and the measurement of wet-to-dry weight ratio, corrected for the content of blood as described by Pearce et al. [10]. Airway obstruction score was measured by methods described by Cox et al. [11]. About 400–500 mg tissue samples of the bronchi (1-2, 2–4 mm) were obtained from the left caudal lobe for microsphere measurements as described.

Data analysis

All values are expressed as mean ± SEM. Outcome variables for physiological parameters were analyzed using Scheffe's *post hoc* test, and $p<0.05$ was considered as statistically significant.

Results

Plasma carboxyhemoglobin level after smoke inhalation

All animals survived the 48-h experimental period. The arterial carboxyhemoglobin levels measured immediately after smoke exposure were similar in the injury + ligation group (60± 4.4% mean ± SE) and injury + no ligation group (62± 3.5%).

Bronchial blood flow

To ensure that the bronchial artery blood flow was ablated by ligation, we used colored microspheres. The change of blood flow at the bronchi (diameter 2–4 mm [Proximal bronchi] and 1–2 mm [Distal bronchi]) before and 24 h after the ligation operation (0 h) is shown in Fig. 1.1. In the ligation group, the blood flow significantly decreased after the ligation operation (P-bronchi; 57.8± 16.43% from base line, P=0.002; D-bronchi; 66.16± 21.3% from base line, P=0.0066, mean ± SE). There was no significant difference in the no ligation group when compared with the ligation group (P-bronchi, D-bronchi: P=0.2111, 0.2858).

Six h after B & S injury, the blood flow in the bronchi was significantly increased in the injury + no ligation group, as shown in Fig. 1.2. It increased to almost 10 times the baseline levels in both proximal and distal bronchi. Thereafter, the blood

flow mildly decreased but never reached baseline level. The blood flow of the injury + ligation group did not increase during the 48-h study period.

Pulmonary transvascular fluid flux

Pulmonary transvascular fluid flux was evaluated by measurement of lung lymph flow. The change in pulmonary transvascular fluid flux is shown in Fig. 2. Some sheep's lung lymph was accidentally stopped, and the number of sheep was different in this data set (Injury + ligation group: n=8; Injury + no ligation group: n=9; no injury + ligation group: n=5). The Injury + no ligation group showed significant increases in lung lymph when compared with the no injury + ligation group (lung lymph: P=0.0011). These values were significantly lower in the injury + ligation group (lung lymph: P=0.0002). In a comparison of 0-h measurements, the injury + no ligation group showed a significant difference at 3 h; however, the injury + ligation group showed a significant change at 12 h. As a whole, there was no significant difference between the injury + ligation and No injury + ligation groups (lung lymph, PI: P=0.3852, 0.2031).

Lung wet-to-dry ratio

The change in lung wet-to-dry ratio is shown in Fig. 3. There was a significant increase in the injury + no ligation group ratio compared with the no injury + ligation group ratio. But there was less of a difference in a comparison between the injury +

ligation group and no injury + ligation group. (Injury + no ligation 6.6± 0.7; Injury + ligation 5.1± 0.4; No injury + ligation 4.0± 0.4, mean ± SE).

Airway obstruction score

The changes in airway obstruction score are shown in Fig. 4. B & S injury (Injury + no ligation group) caused a significant obstruction of bronchi (P=0.005) and bronchioli (P=0.0192) compared with those in the no injury + ligation group. As seen in the injury + no ligation group, the ligation of the bronchial artery significantly reduced the airway obstruction. There was no significant difference between the no injury + ligation group and injury + ligation groups in bronchi (P=0.1364) or bronchioli (P=0.4068) obstruction scores.

Peak and pause airway pressure

The changes in peak and pause airway pressure are shown in Fig. 5. In the injury + no ligation group, both peak and pause airway pressure were markedly increased compared with those seen in the no injury + ligation group. There were statistically significant differences found at 36, 42 and 48 h in peak airway pressure, and at 42 and 48 h in pause airway pressure between the two groups (injury + no ligation group and no injury + ligation group). Reduction of the bronchial blood flow ameliorated these changes. The values were significantly different between the injury + no ligation group and injury + ligation groups at 42 and 48 h in peak airway pressure and 48 h in pause airway pressure.

Gas exchange and shunt fraction

The change in gas exchange and shunt fraction is shown in Fig. 6. B & S injury caused a progressive fall in PaO_2/FiO_2 ratio in the injury + no ligation group. The ratio decreased below 200, a characteristic of ARDS, 30 h after injury.

In the injury + ligation group, the fall in PaO_2/FiO_2 ratio was not as severe and did not reach the clinically-defined ARDS level. There were significant changes at the 36-h time point compared to the injury + no ligation group.

Burn and smoke injury significantly increased pulmonary shunt fraction (Qs/Qt) in the injury + no ligation group compared with the no injury + ligation group ($P=0.0088$). This increase in pulmonary shunt fraction was markedly reduced by bronchial artery ligation. There was no significant difference between the injury + ligation group and no injury + ligation groups ($P=0.1711$).

Cardiopulmonary hemodynamics

The changes in cardiopulmonary hemodynamics are summarized in Table 1. A large amount of fluid was rapidly administered, which decreased the hematocrit of the no injury + ligation group. In contrast to the no injury + ligation group, the injury + no ligation group showed a significant increase in hematocrit at 12, 24 and 48 h post-injury. Heart rate in the injury + no ligation group significantly increased at 24 h post-injury compared to that of the no injury + ligation group. Left atrial pressure (LAP) significantly decreased at 12 h in the injury + no ligation group in relation to the no

injury + ligation group. In addition, hematocrit, heart rate, and LAP increased in the injury + ligation group, but no significant differences were found between the injury + ligation and injury + no ligation groups.

Fluid balance

The change in fluid balance is shown in Table 2 as a Net fluid balance ((water input - urine output)/ body weight). There were fluid balance increases in both B & S injured groups. There was no significant difference between the injury + no ligation and injury + ligation groups.

Discussion

Previously our laboratory group showed immediate airway hyperemia, in which there is a 20-fold increase in bronchial blood flow, using a B & S injured ovine model [3]. Increased bronchial circulation plays a significant role in the spread of injury from the airway to the parenchyma [12]. Moreover, damage to the airway is characterized by mucosal hyperemia and increased airway microvascular permeability, exfoliation of the epithelial lining, mucous secretion, and an acute inflammatory cell influx. These reactions contribute to the formation of obstructive airway casts. We also showed that airway obstruction is an important factor in causing decreased respiratory function in the case of burn and smoke inhalation injury [11].

Sakurai et al. reported that a reduction of bronchial circulation dramatically attenuated the degree of inhalation injury in a 24-h period in an ovine smoke inhalation injury model [13]. Other investigators have shown that bronchial circulation may play a major role in pulmonary and airway edema after smoke inhalation injury when using a 4-h ovine smoke inhalation model [14]. However, in both of these studies, the animals did not receive a burn injury. Smoke inhalation is a serious medical problem and a major cause of morbidity and mortality in thermally injured patients. With the combined burn and smoke inhalation injury, the lung lesion is more severe than with either injury alone [15].

Sakurai et al. also conducted a ligation study using only a smoke-inhalation insult model. We were able to reconfirm the effect of our bronchial artery ligation in the combined burn and smoke injury model. Our data for the injury + no ligation group showed a significant difference at 3 h in lung lymph flow and at 12 h in P/F ratio (in relation to the 0 h time point), but in Sakurai et al.'s study this difference occurred more slowly (at 8 h and 16 h, respectively) [13]. Other data also supported the same tendency. Soejima et al. showed that the damage of combined burn and smoke injury was more severe than smoke injury alone. They speculated that from a burn insult, vasoactive mediators were released and contributed to the alteration of vascular permeability and vasoconstriction immediately after burn injury. These mediators may be inactivated by the administration of large amounts of fluid [15]. Nonetheless, if a smoke inhalation insult was administered, then there was also an increase in microvascular pressure and permeability to both large (e.g., proteins) and small (e.g., electrolytes, water, glucose) molecules [16, 17].

Increased exudation of plasma into the airway plays a role in the formation of the obstructive cast. The cast was produced from an exfoliation of bronchial epithelial lining, mucous secretion, and an acute inflammatory cell influx such as neutrophils into the airway [11]. Obstructive casts contribute to the decrease in pulmonary function after smoke inhalation injury, and may cause atelectasis, pneumonia, and

barotraumas [18]. These casts can lead to respiratory distress in patients with severe inhalation injury.

Murakami et al. advocate that ventilator-induced baro/volume trauma is another mechanism of high airway pressure. Some parts of the lung were obstructed by cast formation, while other parts of the lung were damaged by the ventilator, resulting in a more pronounced change in damage [18].

Obstruction score is a parameter of airway cast formation advocated by Cox et al. [11]. Our present study showed that the obstruction score of the injury + no ligation group was significantly increased compared with that of the no injury + ligation group. Conversely, the obstruction score of the ligation group was improved. Amelioration of airway pressure also supports this change. The prevention of the bronchial cast both reduced the airway resistance and ameliorated airway pressure. There were significant differences between the injury + no ligation and injury + ligation groups at 42 and 48 h in peak airway pressure and at 48 h in pause pressure.

Cox et al. demonstrated the effects of a reduced bronchial cast by direct heparin infusion to the airway after smoke inhalation injury, leading to improved pulmonary function [19]. Murakami et al. applied aerosolized recombinant human antithrombin in combination with heparin directly to the airway to prevent the formation of fibrin clots [20]. Enkhbaatar et al. reported that a combination of anticoagulant therapy with recombinant human antithrombin (rhAT) and heparin in an

aerosolized form prevented airway fibrin formation [21]. Also, for direct lyses of already formed clots, Murakami et al. reported that the use of a fibrinolytic agent (a tissue plasminogen activator) effectively reduced the size of the obstructive airway cast and ameliorated the grade of acute lung injury following burn and smoke inhalation injury [22]. They also used a continuous venous injection of BBS-2 (a selective iNOS inhibitor) to show that the reduction of airway pressure resulted in improved lung function [23]. Additionally, 7-nitroindazole (7-NI), an nNOS inhibitor, exhibited lower levels of iNOS mRNA in a burn and smoke inhalation injury model [24]. This suggests that reducing nNOS levels may decrease iNOS and result in a reduction of airway blood flow in a combined B & S injury ovine model.

One additional marked phenomenon in the present study is the flux in cardiopulmonary hemodynamics and fluid balance. In our study, hematocrit significantly increased in the injury + no ligation group compared to the no injury + ligation group 12 h after injury, and there was a significant increase in heart rate at 24 h. Fluid balance showed no significant difference but did demonstrate a tendency of the plus balance in the injury + no ligation group. The injury + ligation group had a slightly better result than this. Hematocrit and heart rate in the injury + ligation group showed no significant difference from those of the no injury + ligation group. When the other data are considered in tandem, i.e., lung lymph permeability index and lung wet-to-dry ratio, they suggest a significant hypovolemia in the injury + no ligation

group due to lung edema. Soejima et al. speculated that burn wounds release vasoactive mediators that contribute to the alteration of vascular permeability and vasoconstriction immediately following thermal injury, and result in large amounts of fluid loss from the circulation to the systemic interstitial space [15]. Sakurai et al. showed that after severe burn injury, subcutaneous water content increased by more than four times the baseline level [9]. In our present study, there is almost certainly an increase in subcutaneous edema that will also increase the severity of dehydration.

In spite of these conditions, the injury + ligation group ameliorated the edema formation of lung and prevented fluid loss from the circulating plasma. With regard to inhalation injury, it is known that reactive nitrogen species (RNS) and reactive oxygen species (ROS) are formed and mediate tissue damage [23, 25]. The formation of RNS is associated with activation and up regulation of iNOS. Soejima et al. demonstrated that the inhibition of iNOS resulted in a reduction of pulmonary edema and an amelioration of the P/F ratio following combined burn and smoke inhalation injury [26]. We did not measure NOS or 3NT, an index of RNS, but the change of edema and cardiopulmonary hemodynamics is very likely the result of RNS or ROS formation by the lung injury itself. A decrease in bronchial circulation cannot eliminate the inhalation injury, but can prevent edema and cast formation.

The present study demonstrated that the reduction of bronchial blood flow reduced bronchial exudation and airway cast formation, resulting in an ameliorated severity of lung injury caused by combined burn and smoke inhalation injury. Furthermore, during acute lung injury, neutrophils adhere to the injured capillaries and macrophages secrete cytokines, interleukin 1, 6, 8, 10, and tumor necrosis factor α (TNF-α), which stimulate chemotaxis and activate other neutrophils [27]. There is a possibility that the ligation of the bronchial artery reduces the spread of these factors and prevents the chemotaxis of inflammatory cells. We did not find any significant difference in the number of lung PMN cells, but further investigations are needed to elucidate the full effects of reduced bronchial artery blood flow.

We adapted the method of bronchial artery ligation to produce a reduction of bronchial artery blood flow. There are other researchers who have also succeeded in reaching similar conclusions using chemical products; however, those methods have not yet been directly applied to clinical practice, including the ones used in our ligation study. We are expecting some pharmacological compounds such as BBS-2, a selective iNOS inhibitor, and 7NI, a nNOS inhibitor, to be found to reduce the bronchial circulation or bronchial blood flow, which may likely ameliorate the severity of lung pathologies produced by insults such as combined burn and smoke inhalation injury.

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ABBREVIATIONS

ARDS—acute respiratory distress syndrome

MOF—multiple organ failure

ALI—acute lung injury

SEM—standard errors mean

PI—permeability index

NOS—nitric oxide synthase

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Fig. 1.1.

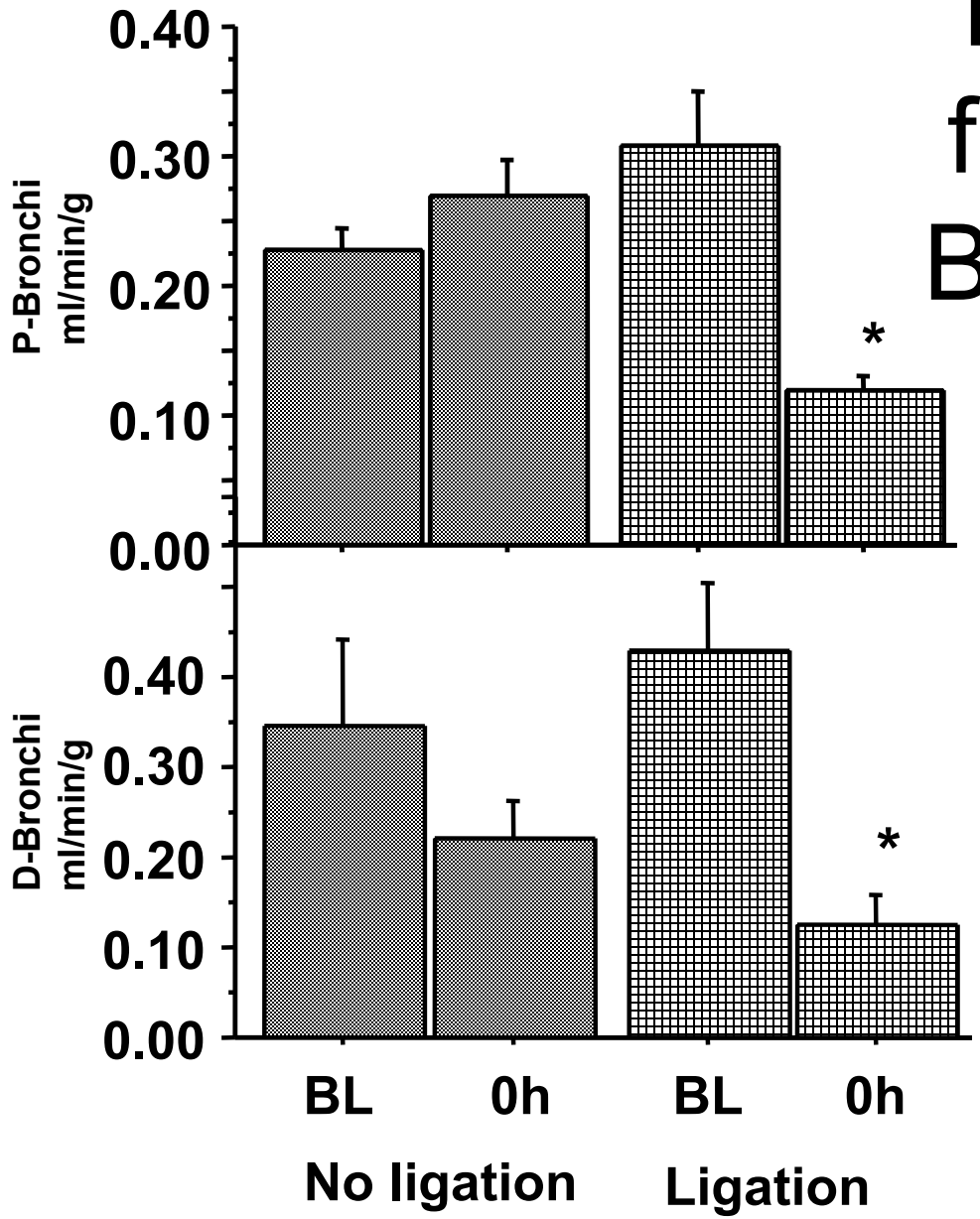


Fig. 1.1. Blood flow of bronchi. Before and after ligation

* p<0.05 vs. BL

Fig. 1.2.

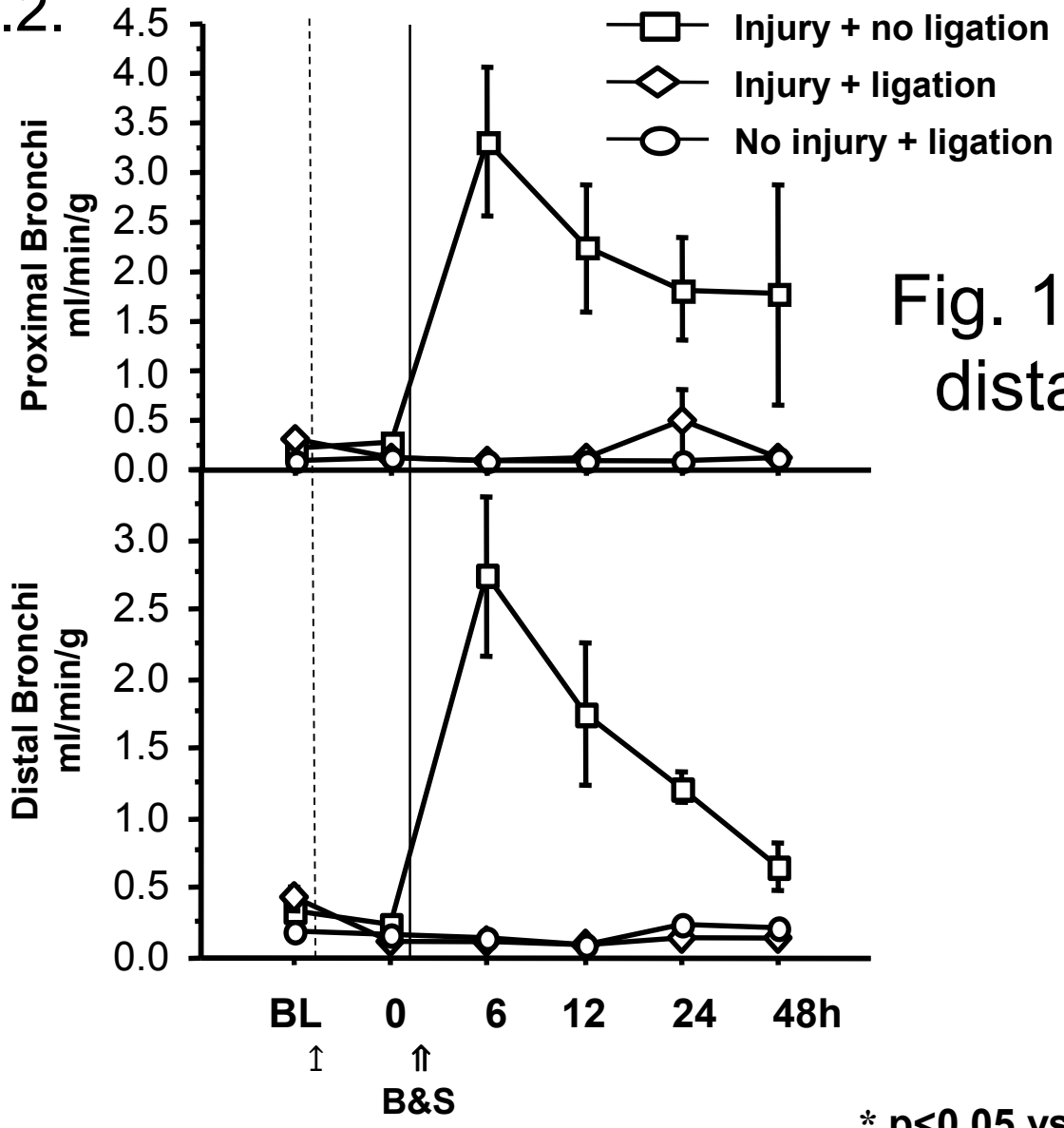


Fig. 1.2. Blood flow of distal and proximal bronchi

* p<0.05 vs. Injury + no ligation

Fig. 2

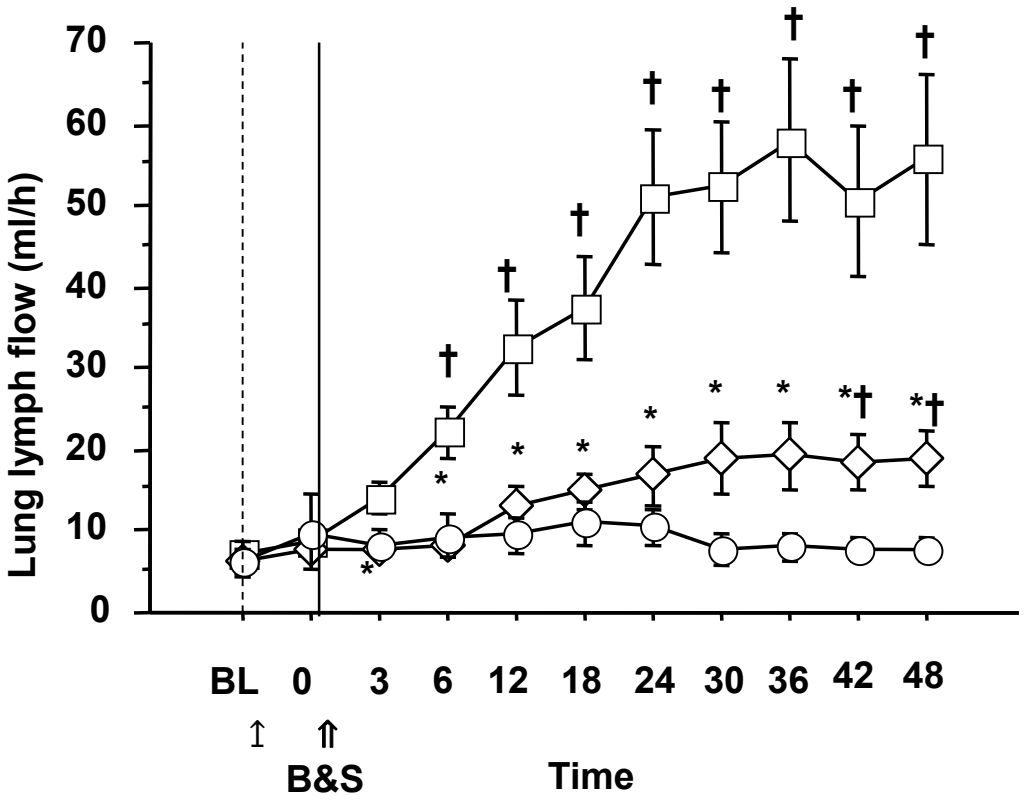


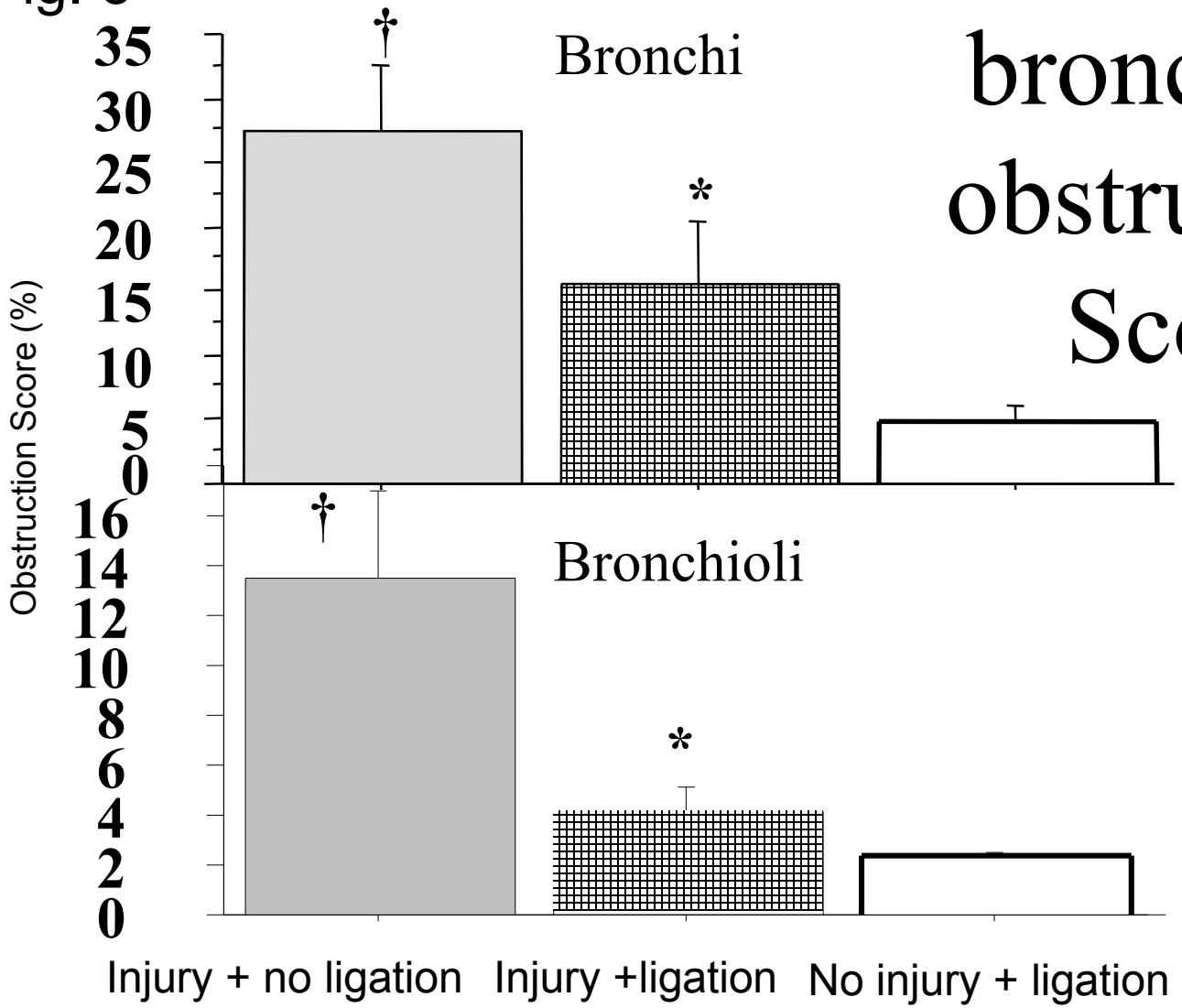
Fig. 2 Lung lymph flow

Injury + no ligation
Injury + ligation
No injury + ligation

* p<0.05 vs. Injury + no ligation
† p<0.05vs. No injury + ligation

Fig. 3

Fig. 3 Bronchi and bronchioli obstruction Score



*** p<0.05 vs. Injury + no ligation**
† p<0.05 vs. No injury + ligation

Fig. 4

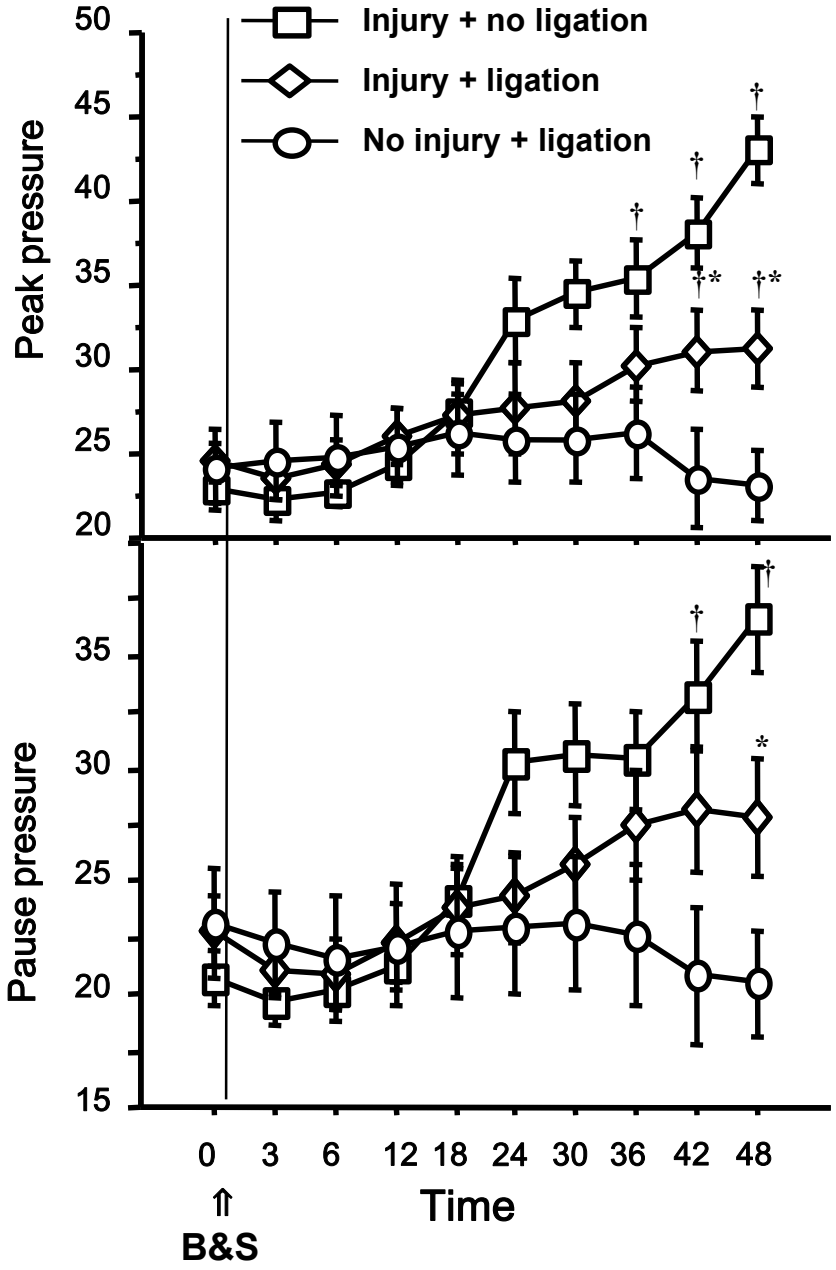


Fig. 4 Peak and Pause pressure

*** p<0.05 vs. Injury + no ligation**
† p<0.05 vs. No injury + ligation

Fig. 5

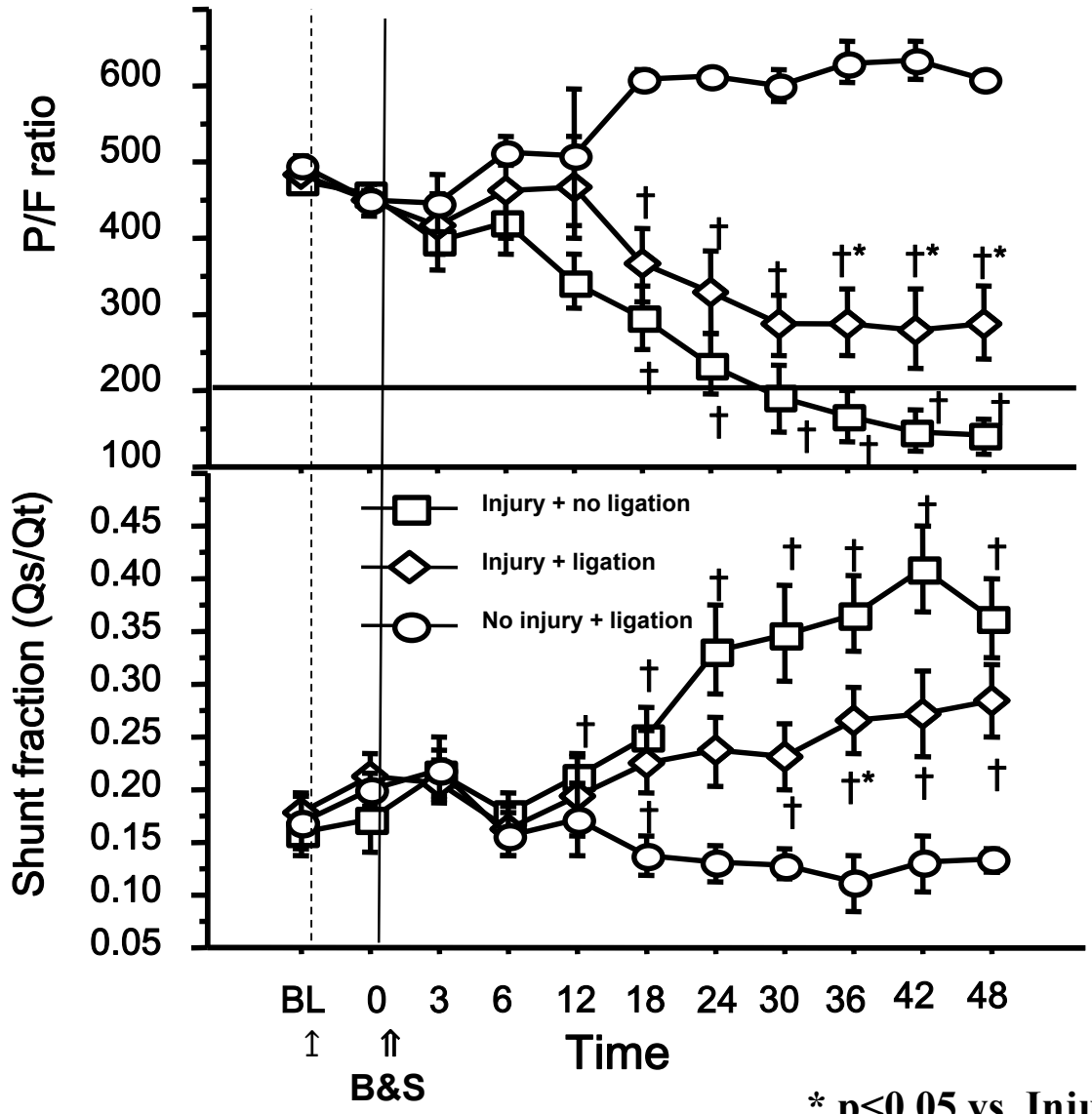


Fig. 5
P/F ratio

*** p<0.05 vs. Injury + no ligation**
† p<0.05 vs. No injury + ligation

Table 1. Cardiopulmonary hemodynamics

	Interval			
	Base line	12h	24h	48h
CO				
Injury + ligation	5.5 +/- 0.3	5.2 +/- 0.3	5.4 +/- 0.2	5.6 +/- 0.2*
Injury + no ligation	5.8 +/- 0.2	5.4 +/- 0.2	5.6 +/- 0.3	6.1 +/- 0.3
No injury + ligation	5.6 +/- 0.2	5.4 +/- 0.4	5.1 +/- 0.2	4.9 +/- 0.4
MAP, mmHg				
Injury + Ligation	102 +/- 3	111 +/- 2 *	113 +/- 5 *	112 +/- 5
Injury + no ligation	101 +/- 3	107 +/- 6	107 +/- 7	105 +/- 4
no injury + ligation	97 +/- 4	99 +/- 6	95 +/- 5	97 +/- 6
PAP, mmHg				
Injury + ligation	20 +/- 1	28 +/- 1	29 +/- 1	28+/- 2
Injury + no ligation	21 +/- 1	25 +/- 1	28 +/- 2	29+/- 2
No injury + ligation	22 +/- 2	27 +/- 2†	27 +/- 2	26+/- 2
LAP, mmHg				
Injury + ligation	7 +/- 1	10 +/- 1 *	11 +/- 1	10 +/- 1
Injury + no ligation	9 +/- 1	8 +/- 1	9 +/- 1	9 +/- 1
No injury + ligation	9 +/- 2	12 +/- 2 †	11 +/- 2	10 +/- 1
CVP, mmHg				
Injury + ligation	6 +/- 1	9 +/- 1	9 +/- 1	9 +/- 2
Injury + no ligation	6 +/- 0	10 +/- 2	9 +/- 1	7 +/- 1
No injury + ligation	6 +/- 2	10 +/- 1	11 +/- 2	9 +/- 1
HR				
Injury + ligation	98 +/- 4	122 +/- 7	110 +/- 7 *	118 +/- 6
Injury + no ligation	106 +/- 5	124 +/- 6	135 +/- 5	123 +/- 7
No injury + ligation	96 +/- 5	105 +/- 7	110 +/- 8†	101 +/- 7
Hct				
Injury + ligation	27 +/- 1	27 +/- 1*	26 +/- 1	26 +/- 2
Injury + no ligation	26 +/- 1	29 +/- 1	29 +/- 1	27 +/- 1
No injury + ligation	27 +/- 2	23 +/- 1	23 +/- 1†	22 +/- 1†

*** p<0.05 vs. Injury + no ligation, † p<0.05 vs. No injury + ligation**

Table 2. Net Fluid Balance

	Interval			
	3h	12h	24h	48h
Net Fluid balance (ml/kg/h)				
Injury + ligation	+16.243 +/- 2.347	+55.489 +/- 3.303	+67.994 +/- 5.705	+56.121 +/- 12.828
Injury + no ligation	+16.431 +/- 2.268	+56.461 +/- 9.899	+81.955 +/- 19.371	+89.324 +/- 26.619 *
No injury + ligation	+8.414 +/- 2.828	+9.037 +/- 7.141†	-4.133 +/- 12.578†	-26.599 +/- 7.837†

*** p<0.05 vs. Injury + no ligation, † p<0.05 vs. No injury + ligation**