Objective: The purpose of this study was to investigate the effects of cardiopulmonary bypass and ultrafiltration on graft function in a canine single-lung transplantation model.

Methods: Fifteen left single-lung transplantations were done in weight-mismatched canine pairs. The animals were divided into 3 groups: group 1, in which transplantation was done without cardiopulmonary bypass; group 2, in which transplantation was done with cardiopulmonary bypass and in which the cardiopulmonary bypass flow was decreased slowly with controlled pulmonary artery pressure; and group 3, in which transplantation was done with cardiopulmonary bypass and ultrafiltration. Hemodynamic parameters and lung function were monitored for 6 hours after reperfusion. The grafts were harvested for histologic studies, myeloperoxidase assay, and real-time quantitative reverse transcription–polymerase chain reaction of mRNA encoding interleukin 6.

Results: The hemodynamic parameters were similar among the 3 groups. In group 1 PaO2 and alveolar to arterial gradient for O2 levels were excellent throughout the 6-hour observation period, but in group 2 they progressively deteriorated. However, ultrafiltration significantly \( P < 0.02 \) improved the PaO2 level in group 3. On histology, interstitial edema and polymonuclear cell infiltration were most marked in group 2 and significantly worse than in groups 1 and 3. Myeloperoxidase assay and real-time quantitative reverse transcription–polymerase chain reaction showed increased myeloperoxidase activity and interleukin 6 gene expression in group 2 grafts compared with group 1 grafts. Myeloperoxidase activity and interleukin 6 gene expression were suppressed with ultrafiltration.

Conclusions: Cardiopulmonary bypass had negative effects on the graft, but ultrafiltration attenuated acute lung dysfunction by reducing the inflammatory response.

The use of cardiopulmonary bypass (CPB) is limited to a minority of lung transplant recipients because CPB has been associated with inferior early graft function and clinical outcome. Several reports have found that the mortality and morbidity of recipients requiring CPB is higher than that of recipients not requiring CPB.\(^1\)\(^2\)\(^3\) One of the reasons for these findings could be that CPB evokes an inflammatory response that leads to organ failure, including respiratory distress syndrome.\(^4\)\(^5\) In cardiac surgery CPB is well recognized to cause a systemic inflammatory response syndrome that can progress to acute lung inflammation known as postperfusion syndrome.

On the other hand, Triantafillou and colleagues\(^7\) reported that CPB has no deleterious effect on early graft function. In pediatric transplantation most operations are performed with CPB, and good results have been reported. In fact, the Harefield group recommends the routine use of CPB in lung transplantation.\(^8\)
has recently been suggested that ultrafiltration is effective in preventing pulmonary edema and preventing the sudden increase of pulmonary flow during the reperfusion period. In canine single-lung transplantations with CPB, and graft dysfunction is controversial. The reason for a lack of consensus is partially due to the fact that clinical studies can be biased because patients who require CPB have more severe disease. In particular, CPB is mostly needed in patients with pulmonary hypertension, and this group of patients has the highest risk of all patients undergoing transplantation. There have been no randomized controlled studies to address the effects of CPB on the outcomes of lung transplantation, and few experimental studies have specifically examined the effects of CPB on graft function.

Theoretically, CPB has potential benefits, such as providing hemodynamic stability during surgical intervention and preventing the sudden increase of pulmonary flow during the reperfusion period. Thus controlling graft flow with CPB might protect the graft from high-pressure reperfusion injury. Halldorsson and coworkers\(^9\) reported that decreasing the reperfusion pressure prevents pulmonary injury in a piglet model. On the other hand, the Pittsburgh group reported that the use of CPB is associated with early graft dysfunction in a canine experiment.\(^\) Fullerton and colleagues\(^1\) reported that lung transplantation with CPB exaggrates pulmonary vasomotor dysfunction. However, in their experiment the animals were quickly weaned off CPB, and the potential benefit of CPB was not examined. We hypothesized that the effect of CPB on lung function depends on the balance between its deleterious effects caused by an inflammatory response and its beneficial effects caused by decreasing pulmonary flow. To examine the beneficial effects of CPB on graft function, we performed canine single-lung transplantations with CPB, and graft flow was controlled during the early reperfusion period. In addition, we examined whether conventional ultrafiltration (CUF) during CPB attenuates graft dysfunction because it has recently been suggested that ultrafiltration is effective in eliminating the inflammatory mediators responsible for the cytokine syndrome.\(^1\)

### Materials and Methods

Fifteen left single-lung transplantations were done in weight-mismatched pairs of adult mongrel dogs. Dogs weighing from 20 to 21 kg were used as donors, and smaller dogs weighing from 15 to 17.5 kg were used as recipients. The donor/recipient body weight ratio ranged from 1.2 to 1.25. Weight-mismatched pairs of dogs were used because Fujita and associates\(^3\) found that the donor/recipient body ratio had an important effect on early graft function; a donor/recipient body weight ratio of 1.2 or greater was associated with stable graft function.

The animals were divided into 3 groups: group 1, in which transplantation was done without CPB; group 2, in which transplantation was done with CPB and CPB flow was decreased slowly with controlled pulmonary artery pressure; and group 3, in which transplantation was done with CPB and ultrafiltration was used until the termination of CPB.

### Donor Procedure

Anesthesia was induced with intramuscular ketamine hydrochloride (10 mg/kg) and atropine (0.01 mg/kg). Intravenous thiopental sodium (2.5 mg/kg) and pancuronium bromide (0.1 mg/kg) were used to facilitate endotracheal intubation. The animals were ventilated at a tidal volume of 15 mL/kg at a rate of 15 breaths/min by using a volume-limited ventilator (Servo 900B, Siemens-Elmier). Positive end-expiratory pressure was controlled at 5 cm H\(_2\)O; the inspired oxygen fraction was 1.0. Anesthesia was maintained with inhalation of 1% to 2% halothane, and muscular relaxation was obtained with additional pancuronium bromide at a dose of 0.5 mg/kg. An arterial line was inserted into the right femoral artery to monitor blood pressure and arterial blood gases. A venous line was inserted into the right femoral vein to allow infusion of solutions. A left thoracotomy was performed at the fifth intercostal space. The left and right pulmonary arteries, pulmonary vein, and left main bronchus were isolated. Sodium heparin (300 U/kg) was administered through the venous line. A purse-string suture with 5-0 Prolene was placed on the proximal site of the left pulmonary artery, and a catheter was inserted. A vascular clamp was placed on the left atrium, and an incision was made for decompression. After left pulmonary artery clamping, the left lung was selectively flushed with cold modified Euro-Collins solution (50 mL/kg). During this period, the lung was ventilated with 100% oxygen. After flushing was completed, the left main bronchus was stapled, leaving the lung well inflated with 100% oxygen. The left lung was then excised and stored in 4°C Euro-Collins solution until transplantation. During pulmonary artery flushing, donor blood for the recipients’ transfusion was collected into packs containing citrate-phosphate-dextrose solution from the venous line. After harvesting the left lung, the animals were killed by means of an intravenous injection with an overdose of potassium chloride and thiopental sodium.

### Recipient Procedure

Recipient dogs were anesthetized and ventilated, and catheters were inserted as in the donors. A pulmonary artery catheter (5F Swan-Ganz catheter) was placed in the main pulmonary artery...
from the left internal jugular vein to monitor pulmonary artery pressure, central venous pressure (CVP), and cardiac output (CO). With the animals in the right decubitus position, a left thoracotomy was done through the fifth intercostal space. The right pulmonary artery and the right main bronchus were encircled by 1-0 silk strings. Sodium heparin (300 U/kg) was administered systemically, and the activated clotting time was confirmed to be longer than 400 seconds. A catheter was placed into the left atrium through the right accessory pulmonary vein to monitor left atrial pressure (LAP). In group 1 a left pneumonectomy was performed, and then the donor left lung was orthotopically transplanted. In groups 2 and 3 a pediatric arterial cannula was inserted in the distal part of the ascending aorta, and a 2-stage wire-reinforced venous cannula (28F, 38F) was placed in the right atrium by opening the pericardium through a left thoracotomy. The arterial and venous cannulas were connected to the bypass circuits, which consisted of a roller pump, a 2000-mL venous reservoir, ¾-inch tubing, a 40-µm arterial filter, a polystan oxygenator (SAFE MINI, Polycare Ltd), and a heat exchanger. The bypass circuit was primed with 500 mL of lactate Ringer’s solution, 20% mannitol, and sodium bicarbonate. CPB was initiated and maintained at a flow rate of 80 to 100 mL/kg for 2 hours, and rectal temperature was maintained at 36°C. During CPB, the respiration rate was decreased to 6 breaths/min. The left pneumonectomy and lung transplantation were done in the same manner as in group 1. When the animals were weaned off CPB, the CPB flow was decreased slowly so that the newly transplanted lungs were reperfused with a controlled pulmonary artery pressure over a 10-minute period. In all groups transplantation involved about 20 hours of ischemic time. All animals were transfused with donor blood if the hematocrit level decreased to less than 20%. The donor blood was endotoxin free and a negative cross-match. In all groups protamine was administered to neutralize heparin, and the activated clotting time was normalized. After 30 minutes of reperfusion, the native right pulmonary artery and right main bronchus were ligated to ensure dependence on the transplanted lung. The lungs were ventilated with a tidal volume of 15 mL/kg, a positive end-expiratory pressure of 5 cm H2O, and an inspired oxygen fraction of 1.0 at a rate of 20 breaths/min. The skin was closed, and the recipient dogs were observed for 6 hours, during which time aortic pressure, pulmonary artery pressure, CVP, and LAP values were continuously monitored. Measurement of CO, arterial blood gas analysis, and blood sampling was done at 60, 120, 180, 240, 300, and 360 minutes after ligation of the right pulmonary artery and the right bronchus. CO was measured by means of the thermodilution technique with a 5-mL bolus injection of 5% glucose at 4°C. Arterial gas was analyzed with the i-STAT portable clinical analyzer (Heska Corp). Bronchoalveolar lavage (BAL) was done twice by means of flexible bronchoscopy, once before transplantation and again 360 minutes after ligation of the right pulmonary artery and the right bronchus. The bronchoscope was wedged in the lingual segment of the left upper lobe. The BAL fluid was collected by flushing the distal airways with 2 × 10 mL of normal saline and consequent intermittent suctioning. After 6 hours of observation, the animals were killed with an intravenous injection of an overdose of potassium chloride and thiopental sodium.

Ultrafiltration
In group 3 the ultrafiltration circuit was arranged as follows. One of the outlet ports of the arterial line was connected to a hemofilter (Capiox CX-HC05S, Terumo Corp) through a small roller pump. A polysulfon fiber with a surface of 0.5 m², a priming volume of 34 mL, and a maximal transmembranous gradient of 500 mm Hg was used. Ultrafiltration was performed at 200 mL/min, and the blood was returned to the venous reservoir after filtration. Ultrafiltration was started 20 minutes before the reperfusion of the transplanted lung and continued until the termination of CPB. A total of 2000 mL of normal saline was infused into the venous reservoir, and the priming volume, including the additional infusion, was completely removed.

Animal Care
All animals received humane care in compliance with the “Principles of laboratory animal care” formulated by the National Society of Medical Research and the “Guide for the care and use of laboratory animals” prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996. The institutional review committee approved the study protocol.

Measurement of Wet/Dry Lung Weight Ratio
The left upper and lower lobes were used to measure the wet/dry weight (W/D) ratio. The lungs were weighed and then dried in an oven at a constant temperature of 80°C for 48 hours. The W/D ratio was obtained by dividing the wet weight by the final dry weight.

Histopathologic Studies
Lung specimens harvested at the time of animal death were fixed with 10% formalin. The tissues, cut into 4-µm sections, were stained with hematoxylin and eosin and with naphtol AS-D chloroacetate esterase. Polymorphonuclear neutrophils (PMNs) were identified by means of positive staining and were counted at 400× magnification by 2 investigators who were blind to the specimens. The data were expressed as PMNs per alveolus.

Myeloperoxidase Assay
The recipient lung samples were frozen in liquid nitrogen and stored at −80°C until assay. Quantitative myeloperoxidase (MPO) activity was determined as previously described.16 Results were expressed as MPO per minute per 1 mg of tissue corrected for dry weight (change in optical density per minute per milligram).

Statistical Analysis
Data were expressed as means ± standard deviation. Data for multiple observations over time were analyzed by means of 2-way analysis of variance (ANOVA) with repeated measures for overall treatment effect. One-way ANOVA was used to test for differences among treatment groups. When ANOVA showed significance, the Tukey test was used for post-hoc multiple comparisons between treatment groups. All analyses were performed with SPSS 13.0J (SPSS Japan Inc) and SAS 8.2 (SAS Institute Inc) software.
TABLE 1. Characteristics of the 3 experimental groups

| Characteristic                     | Group 1       | Group 2       | Group 3       |
|------------------------------------|---------------|---------------|---------------|
| Donor weight (kg)                  | 20.4 ± 1.3    | 19.6 ± 2.1    | 19.9 ± 1.9    |
| Recipient weight (kg)              | 16.6 ± 1.1    | 16.1 ± 3.2    | 15.9 ± 2.8    |
| Ratio of donor/recipient weight    | 1.22 ± 0.01   | 1.24 ± 0.17   | 1.25 ± 0.04   |
| Cold ischemic time (min)           | 1212 ± 13     | 1188 ± 58     | 1197 ± 11     |
| Warm ischemic time (min)           | 62.2 ± 9.3    | 62.6 ± 7.1    | 70.6 ± 9.6    |
| Oxygenation capacity (PaO2:FIO2)   | 564.8 ± 47.1  | 608.7 ± 46.1  | 598.5 ± 38.3  |
| Heparin use (mL)                   | 4.9 ± 0.3     | 4.8 ± 1.0     | 4.8 ± 0.8     |
| Body temperature (°C)              | 35.9 ± 0.6    | 35.8 ± 0.9    | 35.7 ± 0.7    |

FIO2, Fraction of inhaled oxygen.

Results

There were no differences among the 3 groups with respect to donor/recipient body weight ratio, cold ischemic time, warm ischemic time, implantation time, body temperature, and the amount of heparin and protamine used (Table 1). All recipient animals survived the 6-hour observation period.

Lung Function and Hemodynamic Changes

A comparison of the 3 groups and the differences among the 3 groups for every measurement of arterial oxygen and alveolar to arterial gradient for O2 are shown in Figure 1. The PaO2 level was excellent in the animals undergoing transplantation without CPB during the 6-hour observation period. However, in animals undergoing transplantation with CPB, the PaO2 level progressively deteriorated, and significant differences were found among the 3 groups (overall group effect, *P* = .001; group 1 vs group 2, *P* = .001; group 2 vs group 3, *P* = .028). The PaO2 level of the animals undergoing transplantation with CPB was better with the use of ultrafiltration during the early reperfusion period. The alveolar to arterial gradient for O2 of the animals undergoing transplantation without CPB was significantly better than that of the animals undergoing transplantation with CPB (overall group effect, *P* = .003; group 1 vs group 2, *P* = .002; group 2 vs group 3, not significant). The hemodynamic data of the recipients during the 6-hour observation period are shown in Table 2. All 3 groups had similar mean aortic pressure, CVP, LAP, and CO values. The animals undergoing transplantation with CPB and ultrafiltration had significantly lower mean pulmonary artery pressures than the animals undergoing transplantation without CPB (overall group effect, *P* = .012; group 1 vs group 3, *P* = .011). Although the *P* value of the overall group effect for PVR was not significant (*P* = .068), the PVR of animals undergoing transplantation with CPB and ultrafiltration was lower than that of animals undergoing transplantation without CPB at 4 and 6 hours after ligation of the right pulmonary artery and bronchus. Regarding aerodynamic parameters, peak airway pressure was essentially the same among the 3 groups.

Histologic Findings

The microscopic findings for each group are shown in Figure 2. Neutrophil infiltration into the interstitial and alveolar spaces was observed in all groups. These changes were most marked in the animals undergoing transplantation with CPB. The mean PMN infiltration number per alveolus was 6.0 ± 1.2 in group 1, 13.2 ± 2 in group 2, and 4.2 ± 1.3 in group 3. PMN infiltration was significantly higher in group 2 than in the other 2 groups (*P* < .001). Severe perivascular hemorrhage, in addition to alveolar exudates and hemorrhage, was seen in animals undergoing transplantation with CPB. Ultrafiltration attenuated these histologic changes.
The MPO activity of the graft was 0.32 ± 0.02 min/mg for animals undergoing transplantation without CPB, 0.41 ± 0.04 min/mg for animals undergoing transplantation with CPB, and 0.16 ± 0.04 min/mg for animals undergoing transplantation with CPB and ultrafiltration. The MPO activity of the lungs transplanted with CPB was higher than that of the lungs transplanted without CPB (P = .04). Simultaneous use of CPB and ultrafiltration significantly suppressed MPO activity (P = .02).

The W/D ratio of the transplanted lung after 6 hours was 7.49 ± 1.05 for animals undergoing transplantation without CPB, 8.71 ± 1.20 for animals undergoing transplantation with CPB, and 7.37 ± 0.40 for animals undergoing transplantation with CPB and ultrafiltration. There were no statistically significant differences among the 3 groups in recovery rates and total cell numbers in the BAL fluid. The protein concentration of the BAL fluid obtained 6 hours after reperfusion was 0.16 ± 0.05 mg/dL for animals undergoing transplantation without CPB, 0.40 ± 0.16 mg/dL for animals undergoing transplantation with CPB,
and 0.12 ± 0.04 mg/dL for animals undergoing transplantation with CPB and ultrafiltration. The protein level of the animals undergoing transplantation with CPB and ultrafiltration was lower than that of the animals undergoing transplantation with CPB (P = .02), whereas the serum protein levels of the 3 groups were essentially the same (2.53 ± 0.26 mg/dL for group 1, 2.45 ± 0.54 mg/dL for group 2, and 2.40 ± 0.54 mg/dL for group 3).

Interleukin 6 (IL-6) mRNA expression in the lung is discussed in Appendix E1 and Figure E1.

**Discussion**

The use of CPB in lung transplantation remains controversial because clinical and experimental studies have reported different conclusions. CPB is known to stimulate and release several mediators, including complement factors. At the time of reperfusion, the blood activated by CPB goes into the newly transplanted lung, and reperfusion injury could be more severely accelerated compared with cases in which the blood is not activated. On the other hand, CPB has a beneficial effect in that the transplanted lungs can be reperfused with controlled pulmonary artery pressure. Several studies have demonstrated that decreasing the reperfusion pressure reduces lung injury after lung ischemia. Therefore one could expect that CPB should have beneficial effects by preventing high pulmonary flow during reperfusion of the transplanted lung.

In the present experiment all animals survived the 6-hour observation period, which is in contrast to other reports in which animals could not tolerate contralateral pulmonary artery clamping for longer than 2 hours. The reason for the differences in survival could be the different reperfusion methods. In previous canine experiments the animals were quickly weaned off CPB, and the potential benefit of CPB was not fully examined. We believe that that the decreasing of the pulmonary pressure in our model ameliorated graft function to some extent. However, our data indicated that even if pulmonary flow was controlled during reperfusion, pulmonary function eventually deteriorated over the 6-hour observation period, and the graft was not permanently protected from the adverse effects of CPB.

Our study has several limitations. First, the animals underwent transplantation with unilateral lungs and vascular beds that were smaller than in bilateral lung transplantation. However, a donor/recipient body weight ratio of 1.2 or greater was used in this study. Second, canines are thought to be more susceptible to CPB than human subjects. Third, most of the animals undergoing transplantation with CPB required blood transfusion, whereas the animals undergoing transplantation without CPB did not. This might have accelerated the worsening of graft function. Halldorsson and coworkers reported that controlled reperfusion prevents pulmonary injury after 24 hours of lung preservation. In their experiment white blood cells were depleted through a leukocyte-depleting filter. Bando and associates reported similar results in lung injury associated with CPB. They showed that leukocyte depletion improves lung injury after CPB. In our experiment leukocyte depletion was not ap-

![Figure 2. The histopathologic appearance of the graft from the 3 groups is shown. Group 1, A moderate number of isolated single neutrophils are present, mainly within alveolar capillaries (upper). Perivascular edema is not evident (lower). Group 2, A substantial increase in the number of neutrophils present in the alveolar wall is seen (upper). In addition to diffuse alveolar edema, alveolar exudates, and hemorrhage, perivascular hemorrhage is also observed (lower). Group 3, Neutrophil infiltration is markedly attenuated. There are no alveolar exudates and hemorrhage (upper). There is mild edema but no hemorrhage in the perivascular space (lower).](image)
plied; this could explain why there was graft dysfunction despite the prevention of high-flow vascular injury. In fact, the changes observed on histology and the MPO assay were not those of simple lung edema but the result of accumulation of PMNs into the alveolar space. These changes are compatible with those found in lungs after cardiac surgery with CPB. It is well known that there is an inflammatory reaction to CPB with neutrophil sequestration in the lungs, contributing to microvascular injury and postoperative pulmonary dysfunction. Therefore in our model reperfusion pulmonary injury of the transplanted lung might have been further enhanced by the adverse effect of CPB.

Recently, ultrafiltration techniques have been shown to improve postoperative respiratory and cardiac functions after heart surgery. Furthermore, ultrafiltration has been investigated as a potential therapy for severe acute respiratory syndrome because it removes inflammatory mediators and excess free water. Thus we hypothesized that ultrafiltration could reduce inflammatory mediators and be beneficial for graft function in lung transplantation with CPB. CUF is applied during the rewarming phase of CPB, and modified ultrafiltration (MUF) is performed immediately after cessation of CPB; both methods are used in cardiac surgery. Berdat and associates reported that in pediatric cardiac surgery both CUF and MUF could remove inflammatory mediators. They reported that of the cytokines, IL-6 was removed most effectively and that IL-6 was removed to a greater extent with CUF than with MUF. IL-6 is known to be the key cytokine associated with sequestration of neutrophils in the lung. IL-6 stimulates neutrophilia and thrombopoiesis and induces the synthesis of acute-phase proteins. Sustained increased of IL-6 in the plasma and BAL fluid of patients with adult respiratory distress syndrome have been demonstrated and are negatively correlated with disease outcome and patient survival. In this experiment we used the CUF method. We were not able to show whether the improvement of graft function was brought about by the reduction of IL-6 levels in the present study because the specific cytokine kit for canine IL-6 is not yet commercially available. Therefore instead of measuring serum cytokine levels, we showed that IL-6 mRNA was reduced in canines with ultrafiltration by using real-time reverse transcription–polymerase chain reaction (see Appendix E1 and Figure E1). Another possible mechanism by which ultrafiltration could improve graft function might be due to the reduction of adhesion molecules on recipients’ blood lymphocytes. It is well known that some adhesion molecules are upregulated during CPB and have been associated with the adverse effects of CPB. Grunenfelder and associates reported that ultrafiltration led to a significant reduction in not only cytokines but also adhesion molecules, such as soluble E-selectin and intercellular adhesion molecule 1. Both molecules are responsible for the primary adhesion of neutrophils to the endothelium of the vessels. Thus several mechanisms might contribute to the changes observed in grafts treated with CUF. Given our results, further study to determine the optimal ultrafiltration method for patients undergoing lung transplantation with CPB is warranted.

In conclusion, despite controlling pulmonary flow during the reperfusion period, CPB has deleterious effects on the graft in a canine single-lung transplantation model. Ultrafiltration applied during termination of CPB might improve graft function by reducing the inflammatory response through several mechanisms.

We gratefully acknowledge the expert technical assistance of Jyu-ichi Higuchi with the surgical preparation of the dogs.

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**Appendix E1. IL-6 mRNA expression in the lung**

Lung tissue was collected in tubes containing TRIZOL reagent (Invitrogen Japan K.K.) and stored at −80°C until analysis to isolate total RNA from the lungs. Samples were incubated for 15 minutes at 30°C to permit complete tissue dissociation. Samples were mixed with 0.2 mL of chloroform per 1 mL of TRIZOL and were centrifuged at 12,000g for 15 minutes at 8°C. The aqueous phase was collected and transferred to fresh tubes. The RNA was precipitated by mixing with isopropyl alcohol. After incubation for 15 minutes at 30°C, samples were centrifuged at 12,000g for 10 minutes. The supernatant was removed, and 75% ethanol was added to the RNA pellet, which was then mixed by vortexing and centrifuged at 7500g for 5 minutes at 4°C. Finally, the dried RNA was isolated. Reverse transcription was done with 5 μL of hot-denatured DNA-free RNA, 100 pmol of random hexamer primers (pd5[N6], Amersham Pharmacia Biotech), and 100 U of SuperScript II reverse transcriptase (Roche Diagnostics). The reaction mixture was incubated for 10 minutes at 20°C, 30 minutes at 42°C, and 5 minutes at 37°C. Products were precipitated with NaOAc (0.3 M, pH 6) and 2.5 volumes of ethanol (95%). They were then resuspended in 30 μL of diethylpyrocarbonate-water and stored at −20°C. cDNA was diluted 100-fold with DNAse-free water in a siliconized tube (which had 10 ng/μL MS2 RNA to prevent adherence to the tube wall), and 5 μL was then used for real-time PCR. Real-time reverse transcription–polymerase chain reaction on the LightCycler (Roche Diagnostics) was done with a total volume of 20 μL in the presence of 2 μL of 10× reaction buffer (Taq polymerase, deoxyribonucleoside triphosphates, MgCl₂, SYBR Green, Roche Diagnostics) and 2 μL of cDNA (or water as a negative control, which was always included). MgCl₂ was added to a final concentration of 4 mM, and 1.25 pmol of each oligonucleotide primer (Actin-LF: CCCAAGGCGAAACCGCGAGAGAT, product size, 219 bp; Actin-LR: GTCCTCCAGGCACGAGGAC; IL6-LF: AAGGGGCATCTCCAACATCATCAT, 332 bp; IL6-LR: CTCCTGCAAGGTCAGCAGAAGAC) was added. Real-time PCR was performed in glass capillaries with an initial denaturation step of 30 seconds at 95°C, followed by 40 cycles of 0 seconds at 95°C, 5 seconds at annealing temperature (β-actin, 66°C; IL-6, 62°C), and (product length [bp]/25) seconds at 72°C. At the end of each cycle, the fluorescence emitted by the SYBR Green was measured. After completion of the cycling process, samples were subjected to a temperature ramp (from 5°C above annealing temperature to 95°C at 2°C/s) with continuous fluorescence monitoring for the melting curve analysis. The relative quantification analyses the actual amount of a particular target transcript relative to an internal standard (a housekeeping gene) in the same sample. Results were expressed as the target/internal standard concentration ratio of the sample divided by the target/internal standard concentration ratio of the calibrator.

The average and standard deviation of relative gene expression of IL-6 of normal lungs and the experimental groups’ lungs are shown in Figure E1. Expression of IL-6 was significantly increased in group 2 over that in group 1 (P = .024). Simultaneous use of ultrafiltration suppressed IL-6 (P = .047, group 2 vs group 3).
Figure E1. The average and standard deviation of relative gene expression of IL-6 of normal lungs and the experimental groups.