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A new algorithm for DCD pulmonary graft donation

Leif Pierre

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LUND
UNIVERSITY

Faculty of Medicine

DOCTORAL DISSERTATION

by due permission of the Faculty of Medicine, Lund University, Sweden.
To be defended at Segerfalkssalen, BMC, Lund.

Date December 15th at 13.00 pm.

Faculty opponent
Peter Svenarud

Organization LUND UNIVERSITY Department of Cardiothoracic Surgery Clinica Sciences Author(s) Leif Pierre		Document name DOCTORIAL DISSERTATION
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Abstract <p>The great majority of donated lungs today comes from DBD donors (Donation after brain death). We have 140-160 DBD donors in sweden/year. For several reasons, only 35 % of the lungs from these donors can be used for transplantation. There are several thousands patients that are in need of lung transplantation. For this reason, there is a huge interest in DCD (Donation after circulatory death). There have been ethical and legal issues associated with donation of DCD lungs. Today a limited number of countries use pulmonary grafts from so-called controlled DCD donors. Although, the amount of lungs transplanted from DCD donors is limited.</p> <p>The overall aim with the present thesis was to present an ethical an legal algorithm wich will enable us to use both controlled an uncontrolled DCD donor lungs for transplantation.</p> <p>The aims of the thesis;</p> <ol style="list-style-type: none"> 1. Is it possible to successfully transplant maginal lungs from DBD donors using the EVLP technique? 2. Is it possible to eliminate severe atelectases using the EVLP technique? 3. Is administration of pre-harvest heparin of importance for good pulmonary graft preservation? 4. Does extended EVLP treatment per se further improve pulmonary graft function? 5. Does ventilation <i>in situ</i> after circulatory death protect the pulmonary graft from the negative effects of warm ichemia in the DCD situation? 6. Is it possible to avoid pre-harvest heparin in DCD donors without an increased risk of thrombus formation in pulmonary grafts? <p>Our clinical results show that lungs from human maginal DBD donors can be successfully transplanted after undergoing an EVLP procedure. Administration of pre-harvesting heparin seems to be of no importance for thrombus formation in pig pulmonary grafts as long as the are kept in normothermia. Ventilation in-situ seems to protect the pulmonary grafts from the negative effects of warm ischemia. Severe atelectasis can be eminated using the EVLP (Ex vivo lung perusion). Extended EVLP treatment did not further improve the pulmonary graft function.</p>		
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To my Family

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- 1.** Ingemansson R, Eyjolfsson A, Mared L, Pierre L, Algotsson L, Ekmechag B, Gustafsson R, Johnsson P, Koul B, Lindstedt S, Lührs C, Sjöberg T, Steen S. Clinical transplantation of initially rejected donor lungs after reconditioning ex vivo. *Ann Thorac Surg.* 2009 Jan; 87(1):255-60.
- 2.** Lindstedt S, Pierre L, Ingemansson R. A Short Period of Ventilation without Perfusion Seems to Reduce Atelectasis without Harming the Lungs during Ex Vivo Lung Perfusion. *J Transplant.* 2013; 2013:729286.
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- 4.** Pierre L, Lindstedt S, Hlebowicz J, Ingemansson R. Is it possible to further improve the function of pulmonary grafts by extending the duration of lung reconditioning using ex vivo lung perfusion? *Perfusion.* 2013 Jul; 28(4):322-7.
- 5.** Pierre L, Lindstedt S, Ingemansson R. Ventilation in situ after cardiac death improves pulmonary grafts exposed to 2 hours of warm ischemia. *Scand Cardiovasc J.* 2015; 49(5):293-8.
- 6.** Pierre L, Lindstedt S, Ingemansson R. Protection of pulmonary graft from thrombosis in donation after cardiac death: effect of warm ischemia versus cold ischemia. *Interact Cardiovasc Thorac Surg.* 2016 Jul 5. pii: ivw225. [Epub ahead of print]

Sammanfattning på svenska

Lungtransplantation är idag en etablerad behandlingsmetod vid vissa mycket allvarliga lungsjukdomar [1]. I Lund och på andra etablerade transplantationscentra i världen är 5-årsöverlevnaden efter lungtransplantation nu ca 70 % [2]. Tjugo procent av patienterna som sätts upp på väntelistan för lungtransplantation avlider i väntan på transplantation eftersom bristen på donator organ är mycket stor. Ca 1500 personer/år i Sverige går hemma med permanent syrgasbehandling. I Sverige har vi cirka 160 hjärndöda donatorer varje år [3]. Från dessa kan man använda lungorna för transplantation i cirka 30 % av fallen [4]. I övriga fall uppfyller inte lungorna kriterierna för transplantation eller så ser man tydliga tecken på infektion, malignitet eller annan allvarlig skada i lungorna. Man vet att antalet potentiella DCD (Donation efter Cirkulations Död") lung donatorer från Sverige skulle vara 15-20 000/år. I EU finns ca 375 000 potentiella DCD donatorer/år och i USA 275 000 . [5-10].

Vår forskningsgrupp har arbetat med EVLP (*Ex Vivo Lung Perfusion*) metoden, som är framtagen av professor Stig Steen, vid Lunds Universitet [11]. Med denna metod kan man studera funktionen hos lungor utanför kroppen i en krets med både perfusion och ventilation av lungan. Man kan i vissa fall även förbättra lung funktionen hos initialt ej transplantabla lungor från hjärndöda donatorer. Man har kunnat visa att man med denna metod kunde öka tillgången på acceptabla lungor från hjärndöda donatorer [12, 13].

Detta ger ett betydligt bättre utnyttjande grad av de lungor som idag doneras. Trots detta räcker lungorna inte till för alla som behöver lung transplanteras. Av den anledningen har vi valt att närmare studera och ta fram ett sätt för att möjliggöra användandet av lungor från DCD donatorer.

Vi har gjort stordjursförsök på gris i syfte att kunna använda lungor från DCD donatorer (II-VI). Problemen har varit att man kliniskt i ett tidigt skede efter dödförklaringen har trott att man måste antikoagulera patienten för att förhindra att blodet i lungan skall bilda blodproppar [14]. De vetenskapliga fynden angående frågan om heparin eller inte går isär [15-18]. Dessutom har man behövt lägga ett dränage i respektive lunsäck för att kyla lungan, via insprutning av kall vätska, för att skydda lungan mot ischemi [19]. Detta har visat sig vara etiskt och juridiskt svårt att genomföra i den kliniska situationen då man ofta inte vet den avlidnes eller de anhörigas inställning till donation innan dessa förberedande invasiva interventioner måste påbörjas. Av denna anledning har metoden ej nått sin fulla kliniska potential.

Sammanfattningsvis har vi i våra djurstudier på gris visat att vi kan avstå från att antikoagulera donatorn under förutsättning att man inte kyler lungan, att ventilation av lungan efter döden ger oss extra tid för kontakt med anhöriga och planering och förberedelser inför EVLP och att EVLP är ett viktigt kvalitetsverktyg för att utvärdera funktionen av lungan och i vissa fall förbättra funktionen. Därmed kan vi undvika att vidta förberedande invasiva åtgärder på donatorn innan vi vet donatorns och anhörigas inställning till donation. När patienten dödförklarats och vi fått de erforderliga tillstånden från anhöriga kan vi genom att påbörja ventilation vinna ytterligare tid vilket möjliggör transport till operationsavdelning, steriltvätt och organ uttag mm. Efter rekonditionering och evaluering kan vi ta ställning till om lungorna uppfyller kriterierna för transplantation.

Vi har nu i Lund fått etiska nämndens godkännande att undersöka om vi kan reproducera på människa vad vi visat på gris. Vi har även etiskt tillstånd att transplantera dessa lungor om de uppfyller kriterierna för transplantation.

Summary in English

Lung transplantation is an established treatment for some very serious lung diseases. Lund and other established transplant centers in the world is the 5-year survival after lung transplantation is now about 70%. Twenty percent of the patients who are put on the waiting list for lung transplantation dies awaiting transplant because the shortage of donor organs is very high. About 1500 persons / year in Sweden goes home with permanent oxygen therapy. Sweden has about 160 brain dead donors each year. From these, one can use the lungs for transplantation in approximately 30% of cases. In other cases, do not meet the criteria for lung transplantation or as one sees clear signs of infection, malignancy or other serious damage in lungs. We know that the number of potential DCD (“donation after circulatory death”) lung donors from Sweden would be 15-20000 / year. In the EU there are around 375 000 potential DCD donors / year and in the USA 275 000.

Our research group has been working with EVLP (*ex vivo* lung perfusion) method, which was developed by Professor Stig Steen at Lund University. With this method one can study the function of the lungs outside the body in a circuit with both perfusion and ventilation of the lung. It may in some cases improve lung function in initially not transplantable lungs from brain dead donors. It has been shown that with this method could increase the availability of acceptable lungs from brain dead donors.

This gives a much better utilization of the lungs donated today. Although this is sufficient lungs are not for everyone who needs lung transplant. For this reason we have chosen to study and develop a way to allow the use of lungs from DCD donors.

We have made great animal experiments on pigs in order to be able to use lungs from DCD donors. The problem has been that clinically in the early stages after the confirmation of death is believed to have anti-coagulate the patient to prevent the blood in the lungs to form blood clots, and put a drain in each pleura to cool the lung via the injection of cold fluid to protect the lung against ischemia. This has proven to be ethically and legally difficult to implement in the clinical situation where you often do not know the deceased or the family's attitude towards donation before these preparatory invasive interventions must begin. For this reason, the method has not reached its full clinical potential.

In summary, we have in our animal studies in pigs showed that we can refrain from anti-coagulate the donor on condition that it does not cool the lung, the ventilation of the lung after death gives us extra time for contact with relatives and the planning and preparation for EVLP and EVLP is a important quality tools to assess the function of the lung, and in some cases improve the function. Thus, we can avoid taking preparatory invasive actions of the donor before we know the donor's and the family's attitude towards donation. When the patient is declared dead and we received the necessary permits from relatives, we can begin by ventilation win additional time which allows transport to the operating room, sterile wash and body withdrawals mm. After reconditioning and evaluation, we can decide if the lungs meet the criteria for transplantation.

We are now in Lund received ethics committee approval to investigate whether we can replicate in humans what we found in pigs. We also have ethical permission to transplant the lungs if they meet the criteria for transplantation.

Abbreviations

AP	Airway Pressure
BOS	Bronchiolitis Obliterans Syndrome
CO	Cardiac Output
COPD	Chronic Obstructive Pulmonary Disease
DBD	Donation after Brain Death
DCD	Donation after Circulatory Death
DLTx	Double-Lung Transplantation
ECC	Extra-Corporeal Circulation
ECMO	Extra-Corporeal Membrane Oxygenation
EVLV	Ex Vivo Lung Perfusion
HTC	Hematocrit
HLTx	Heart and Lung Transplantation
ISHLT	International Society of Heart and Lung Transplantation
LA	Left Atrium
LTx	Lung Transplantation
NHBD	Non-Heart-Beating Donor
PA	Pulmonary Artery
PAP	Pulmonary Artery Pressure
PF	Pulmonary Fibrosis
PGD	Primary Graft Dysfunction
PVR	Pulmonary Vascular Resistance
RBC	Red Blood Cell
SLTx	Single Lung Transplantation

Introduction

Background

Today, lung transplantation is an established treatment for end-stage lung disease of various kinds. In 1963, Dr James Hardy performed the first clinical lung transplantation in Jackson Mississippi [20]. The patient was a 58-year-old man with lung cancer who was serving a life sentence and who agreed to the procedure. At that time, modern immunosuppression had not yet been developed. The patient died 18 days after the transplantation. Over the next 10 years, another 36 lung transplantations were performed at different hospitals around the world. Most of the recipients died within days, but two of the 36 recipients lived for more than a month. The major cause of death was poor healing of the bronchial anastomosis. Dr Shumway and his colleagues at Stanford University performed three heart-lung transplantations in 1981 [21]. One patient died four days after the operation due to multiple organ failure. The other two were alive when the report was published in 1982. In 1983, a group in Toronto, Canada, performed their first lung transplantation on a 58-year-old patient with lung fibrosis. The recipient was treated with cyclosporine and azathioprine for immunosuppression. Three years later, he was still alive and living a normal life, according to the Toronto group.

Lung transplantation continues to be hindered by the number of donors available. *Ex vivo* lung perfusion (EVLP) has emerged as an essential tool for the reassessment, under a controlled scenario, of lungs from heart-beating donors (HBDs) who did not meet transplantation criteria initially [22]. The method is also an excellent tool for reassessment of lungs of donors after cardiac death (DCD). The use of DCD lungs has attracted much interest lately. DCDs are classified according to the Maastricht classification and may be subdivided into controlled and uncontrolled [23]. According to Maastricht criteria, controlled donors are defined as those in which cardiac arrest would be expected after the withdrawal of life support, but before they are declared brain dead (Category 3), and those in which cardiac arrest occurred after brain death (Category 4). Uncontrolled DCDs, Categories 1 and 2, are donors who die in the pre-hospital environment or have unexpected cardiac arrest in the hospital. Recently, one more category was added to the Maastricht classification (Category 5). This is when the donor suffers from unexpected circulatory arrest in the ICU. It is classified as an uncontrolled donor situation.

The controlled DCDs are often of interest, since these patients are under hospital care, and their clinical history and lung function are known. It is also logistically easier to handle these donors. These controlled donors are, however, limited in number compared to the potential numbers of uncontrolled DCDs. The disadvantage of using lungs from uncontrolled donors, however, is that lung function is not known and must be evaluated before the lungs can be accepted for transplant. There are also some issues regarding the optimal preservation of uncontrolled donor lungs, such as the length of time of warm ischemia the lungs can withstand and whether it is better to harvest the lungs after the period of warm ischemia or to cool the lungs inside the deceased donor. These issues are relevant for the donation team. The formalities of the donation process must also be managed properly according to the law of each country. As mentioned above, EVLP is also an excellent tool for reassessment of DCD lungs. How to perform the optimal EVLP has also been a subject for discussion [24, 25].

History of *ex vivo* lung perfusion (EVLP)

According to the Thirtieth Adult Lung and Heart-Lung Transplant Report, 2013, from the Registry of the International Society for Heart and Lung Transplantation, lung transplantation (LTx) is a therapy that is being performed worldwide, with numbers increasing every year [26].

In 2011, 3,640 LTx were reported—compared to only 1,712 cases annually a decade ago. As the outcomes tend to improve, an increasing number of patients with end-stage lung disease are being considered for LTx. Nevertheless, the amount of lungs suitable for transplantation has not followed this trend, and this generates considerable waiting list mortality (approximately 15%). Donor lungs are subjected to several injurious mechanisms during the brain death/organ donation process (such as ventilator-acquired pneumonia, neurogenic and hydrostatic pulmonary edema, and barotrauma). Thus, it is not surprising that the majority of donor lungs are not used for transplantation [27].

Isolated organ perfusion was first described by A. Carell and C. Lindberg in the 1930s[28] . The first group to report a clinically useful lung evaluation model was S. Steen et al. at Lund University Hospital in 2001. Since this method was described, several transplantation groups has been trying to refine and implement EVLP as a platform for evaluation, reconditioning, and treatment of lung graft injuries and to increase the donor pole in both DBD and DCD donors .Two main methods are used today in clinical EVLP, the Lund model and the Toronto model. One difference between the two methods is that a cellular evaluation solution is used in the Lund method. Also, in the Toronto method, the left atrium is closed during the perfusion and in the Lund method it is open. In the Lund method, 100% of the donor's estimated cardiac output is circulated through the lungs, as compared to 40% in the Toronto method. Both methods have shown equally good results in lung transplants using marginal or DCD donors. Even though most of the published articles have had small study groups that were usually not randomized, and also limited long-term follow-up, the method is attracting much interest for use as an important tool for assessment of more transplantable lungs, both from marginal donors (initially rejected) and DCD donors [29].

Aim of the thesis

The aim of the work described in this thesis was to address the following questions:

Is it possible to successfully transplant marginal lungs from DBD donors using the EVLP technique?

Is it possible to eliminate severe atelectases using the EVLP technique?

Is administration of pre-harvest heparin of importance for good pulmonary graft preservation?

Does extended EVLP treatment per se further improve pulmonary graft function?

Does ventilation *in situ* after circulatory death protect the pulmonary graft from the negative effects of warm ischemia in the DCD situation?

Is it possible to avoid pre-harvest heparin in DCD donors without an increased risk of thrombus formation in pulmonary grafts?

Materials and methods

Clinical study (I)

In study I, lungs from nine donors were investigated between June 2006 and April 2007 using the Lund EVLP technique. The criteria for lungs to undergo reconditioning were the same as for ordinary donor lungs, except that we accepted lower values of partial pressure of oxygen in arterial blood (PaO_2). All lungs from the nine donors were initially rejected due to bad oxygenation capacity. In one case (death due to intracerebral bleeding), emphysema and fibrosis were found, so reconditioning was never started. For lungs to be accepted for transplantation, we had already decided that the PaO_2 at an inspired oxygen fraction (FiO_2) of 1.0 should be 50 kPa or higher after reconditioning. In two cases (intracerebral bleeding), the PaO_2 was only 38 and 40 kPa and no transplantation was done. Twelve lungs from six donors were reconditioned and accepted for double lung transplantation. Relevant data for these donors are presented in Table 1. Donor no. 1 came from England and the other five were from four different hospitals in Sweden. Donor 4 had an unwitnessed out-of-hospital cardiac arrest; cardiopulmonary resuscitation (CPR) was successful but brain death was later diagnosed, and the next of kin gave permission for organ donation. Donor 5, a 34-year-old woman, suffered cardiac arrest secondary to an epileptic fit in her home 26 km from our hospital. The patient received bystander CPR before LUCAS-CPR (Lund University Cardiopulmonary Assist System) was started and continued for 40 min until the patient achieved spontaneous stable circulation. Four days later, she was declared brain dead and donation was accepted by the next of kin. The other four donors were declared brain dead after intracerebral hemorrhage.

Table 1. The Donors

Variable	Donor:						Median (range)
	1	2	3	4	5	6	
Sex	Female	Male	Female	Female	Female	Male	
Age (years)	63	58	62	52	34	60	59 (34–63)
Height (cm)	158	169	170	173	165	180	170 (158–180)
Weight (kg)	63	94	75	77	55	80	76 (55–94)
Blood group	A+	O+	A+	O+	O+	O+	
CMV	+	+	+	+	—	+	
Cause of death	Cerebral bleeding	Cerebral bleeding	Cerebral bleeding	Cardiac arrest ^a / ROSC	Cardiac arrest ^b / ROSC	Cerebral bleeding	
Smoking	10–15 cig/day 1 year stop	—	40 cig/day	—	15 cig/day	—	
Pao ₂ (kPa) ^c before harvesting (Fio ₂ = 1.0)	28.7	18.2	19.7	11.5	22.4	22.8	21.1 (11.5–28.7)

^a After cerebral bleeding. ^b After epileptic fit. ^c 1kPa = 7.5 mm Hg.

CMV = cytomegalovirus; Fio₂ = fraction of inspired oxygen; Pao₂ = partial pressure of oxygen in arterial blood; ROSC = return of spontaneous circulation (after successful cardiopulmonary resuscitation).

Initial preservation and transportation of the donor lungs

The lungs from donors 2–6 were preserved by cold infusion of the pulmonary artery with Perfadex solution (Vitrolife AB, Kungsbacka, Sweden) with added calcium chloride (0.3 mmol/L), nitroglycerine (5 mg/L), and trihydroxymethylaminomethane buffer (0.3 mmol/L) [10–12]. The infusion (80 mL/kg body weight) was given with low perfusion pressure (15–20 mmHg). At the retrieval operation, a segment (8 cm) of the descending aorta was also excised. The lungs were immersed in cold Perfadex in a semi-inflated state with 100% oxygen together with the aortic segment, and transported to our hospital in a cooling box. For donor 1, Papworth solution was used for the initial preservation.

Ex vivo lung reconditioning

Figure 1 shows the perfusion circuit. Note the prolongation of the pulmonary artery by a segment of the descending aorta, to make the cannulation easier. All hearts (except the dorsal wall of the left atrium with the pulmonary veins) had been retrieved for the purpose of transplantation or homograft harvesting. During the reconditioning, the perfusion solution flowed directly out in the lung reconditioning box, so left atrium pressure was always zero. The extracorporeal perfusion circuit was delivered by Medtronic (Medtronic BV, Kerkrade, the Netherlands; Ex Vivo Lung Evaluation Set). The system was primed with 2 L of STEEN solution (Vitrolife AB) mixed with two units of ABO-compatible erythrocyte concentrate that had been irradiated, leukocyte-filtered, and washed. STEEN solution is a physiological electrolyte solution

containing human serum albumin and dextran, to keep a high colloid osmotic pressure. Imipenem (0.5 g; Tienam; Merck Sharp & Dohme, Sollentuna, Sweden), insulin (20 IU; Actrapid; Novo Nordisk, Bagsvaerd, Denmark), and heparin (10,000 IU; LEO Pharma, Malmö, Sweden) were added, and isotonic trometamol (Addex-Tham; Kabi, Sweden) was used to buffer the mixed solution to a temperature-adjusted pH of 7.4. Gas was supplied to the membrane oxygenator; first oxygen and CO₂ during the reconditioning phase, and then 93% nitrogen and 7% CO₂ during the testing phase, creating a normal venous blood gas in the perfusate to the pulmonary artery (i.e. the oxygenator is used to deoxygenate the perfusate). Before the perfusion was started, the pulmonary artery cannula was connected to the corresponding tube of the extracorporeal circuit, the air was removed, and the shunt of the circuit was clamped (Fig. 1).

A low-flow perfusion at 25°C was initiated through the lungs. They were gradually warmed by increasing the temperature of the perfusate. When the temperature of the perfusate from the lungs stabilized at 32°C, careful ventilation was started. The pump flow was gradually increased, but the pulmonary artery pressure was never allowed to exceed 20 mmHg. After 20–30 min, normothermia was reached. The positive end-expiratory pressure was increased during short intervals to fully expand the lungs and eliminate atelectasis, but it was otherwise kept at 5 cm H₂O. Blood gases were analyzed throughout the perfusion, and when they were acceptable (PaO₂ 20 kPa at an FiO₂ of 0.5), perfusion flow and ventilation were kept constant. For the final evaluation, the erythrocyte-containing perfusate was deoxygenated and entered the lung circulation through the pulmonary artery. The lungs were ventilated with decreasing oxygen fractions and the oxygenation capacity, obtained from analyzing the perfusate leaving the lung circulation, was then calculated. If the blood gases met the criterias for transplantation, the next phase was initiated. This method has been approved by the Ethics Committee of Lund University.

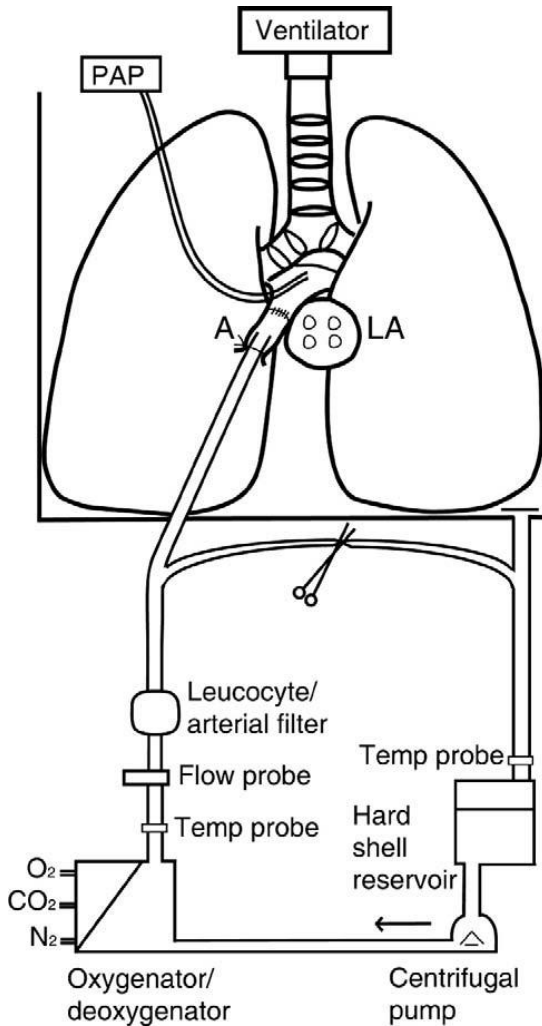


Fig 1.

Schematic drawing of the ex vivo lung reconditioning system. The lungs are placed in the reconditioning box, connected to the per-fusion system and a ventilator. The cannulation is done through a piece of aorta (A), harvested from the donor, and used to prolong the pulmonary artery. The blood coming out from the remaining dorsal part of the left atrium (LA) runs freely out in the box. Pulmonary arterial pressure (PAP) is measured continuously. Blood gases are measured in the blood before and after the lung. The shunt with the tube clamp is used to prime the system with STEEN Solution mixed with erythrocytes to a hematocrit of 10% to 15%.

Topical extracorporeal membrane oxygenation (ECMO)

When reconditioning and evaluation were completed, the temperature of the ingoing perfusate was reduced to 25°C; when it had stabilized, the perfusion was stopped. The pulmonary artery cannula and the trachea were clamped with the lungs in a semi-inflated state (FiO₂ of 1.0). The lungs were then immersed in the perfusate, to which a buffered Perfadex solution was added. The extracorporeal circuit was then used to perfuse the solution in the box containing the immersed lungs, keeping that medium oxygenated and cooled to 8°C.

Transplantation

Double sequential lung transplantation was performed with clam shell incision (4 patients) or bilateral thoracotomy (2 patients), and without the support of extracorporeal circulation, except in recipient 5.

Logistics of the procedures

If the donor lungs arrived after 11 p.m., they were kept in Perfadex solution at 4–6°C and the reconditioning was postponed until 9 a.m. the following day; they were then transplanted. If the reconditioning procedure had not been completed before 11 p.m., successfully reconditioned lungs were stored in topical ECMO at 8°C until the transplantation was started at 9 a.m. the next day.

Experimental animals (II, III, IV, V, VI)

In this thesis, 54 pigs in total were used. The weight of the pigs used was in the 50–70 kg range. The studies were approved by the Ethics Committee for Animal Research, Lund University. The experimental protocol for the studies was approved by the Ethics Committee for Animal Research, Lund University. All animals received care according to the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes, the Principles of Laboratory Animal Care of the National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals.

Preparation of animals (II, III, IV, V, VI)

Pre-medication was performed with an intramuscular injection of xylazine (Rompun® vet., 20 mg/ml; Bayer AG, Leverkusen, Germany; 2 mg/kg) mixed with ketamine (Ketaminol® vet., 100 mg/ml; Farmaceutici Gellini S.p.A., Aprilia, Italy; 20 mg/kg) while the pig was still in its stable. Peripheral i.v. access was then established in the ear. The pig was then transferred to the laboratory and placed on the operating table in the supine position. Oral intubation was performed using a 7.5-mm endotracheal tube after induction of anesthesia with sodium thiopental (Pentothal; Abbott Laboratories, Chicago, Illinois, USA) and pancuronium bromide (Pavulon; N.V. Organon, Oss, the Netherlands). Anesthesia was maintained by infusions of ketamine, midazolam (Midazolam Panpharma®; Panpharma, Oslo, Norway), and fentanyl (Leptanal®; Lilly, France). Fluid loss was compensated for by the continuous infusion of Ringer's acetate at a rate of 300 mL/kg/h. Mechanical ventilation was established with a Siemens-Elema ventilator (Servo Ventilator 300; Siemens, Solna, Sweden) with an inspired oxygen fraction (FiO₂) of 0.5, a frequency of 15 breaths/min, a minute ventilation of 6 l/min, and a positive end-expiratory pressure (PEEP) of 5 cmH₂O.

Presevation of the pulmonary grafts (II)

Ventricular fibrillation was induced electrically. The tracheal tube was disconnected from the ventilator when circulatory arrest was confirmed. The animals were left untouched for 1.5 h at room temperature. Thereafter, a median sternotomy was performed. The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse-string suture placed in the outflow tract of the a. pulmonalis. A clamp was placed on the v. cava superior, and another clamp on the v. cava inferior. A third clamp was then

placed on the ascending aorta. The left atrium and the v. cava inferior were then opened. The right and left pleurae were filled with ice slush to cool the lungs. The lungs were perfused antegradely with 5 L of cold Perfadex containing 1.0 mL isotonic trometamol (Addex-THAM, 3.3 mmol/mL; Fresenius Kabi AB, Uppsala, Sweden), 2 mL calcium chloride (0.45 mmol/mL), and 3 mL nitroglycerine (5 mg/mL; BMM Pharma AB, Stockholm, Sweden) at a low perfusion pressure (< 20 mmHg). The cannula was then removed from the pulmonary artery. The lungs were harvested en bloc in a standard fashion, and weighed. A segment (~8 cm) of the descending aorta was also excised. The lungs, together with the aortic segment, were then immersed in cold Perfadex and kept in cold storage at 6°C for 2 h.

Preservation of HBD lungs (III)

The pigs were randomly assigned to one of two groups: one receiving heparin sodium, and the other not receiving heparin sodium. A median sternotomy was performed. Heparin sodium (Heparin LEO; 400 IE/kg; LEO Pharma AB, Malmö, Sweden) was administered intravenously to one group of animals. The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse-string suture placed in the outflow tract of the a. pulmonalis. A clamp was put on the v. cava superior, and another clamp was put on the v. cava inferior. A clamp was then put on the ascending aorta. The left atrium and v. cava inferior were then opened. The right and left pleurae were filled with ice slush to cool the lungs. They were perfused antegradely with 2 L of cold Perfadex® containing 1.0 mL isotonic trometamol (Addex-THAM, 3.3 mmol/ml; Fresenius Kabi AB Uppsala, Sweden), 2 mL calcium chloride (0.45 mmol/mL), and 3 mL nitroglycerine (5 mg/mL; BMM Pharma AB, Stockholm, Sweden) at a low perfusion pressure (20 mmHg). The cannula was then removed from the pulmonary artery. The lungs were harvested en bloc in a standard fashion, and weighed. A segment (~ 8 cm) of the descending aorta was also excised. The lungs, together with the aortic segment, were then immersed in cold Perfadex and kept in cold storage at 8°C for 4 h.

Preservation of the pulmonary grafts (IV)

A median sternotomy was performed and heparin sodium (Heparin LEO, 400 IE/kg; LEO Pharma AB, Malmö, Sweden) was given intravenously. The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse-string suture placed in the outflow tract of the pulmonary artery. A clamp was put on the caval superior vein and the caval inferior vein was clamped. A clamp was then put on the ascending aorta. The left atrium and v. cava inferior were opened. The right and left pleurae were filled with ice slush to cool the lungs.

The lungs were perfused antegradely with 80 mL/kg of cold Perfadex® containing 1.0 mL isotonic trometamol (Addex-THAM, 3.3 mmol/ml; Fresenius Kabi AB, Uppsala, Sweden), 2 mL calcium chloride (0.45 mmol/mL), and 3 mL nitroglycerine (5 mg/mL; BMM Pharma AB, Stockholm, Sweden), distributed at a low perfusion pressure (< 20 mmHg). The cannula was then removed from the pulmonary artery. The lungs were harvested en bloc in a standard fashion, and weighed. A segment (~ 8 cm) of the descending aorta was also excised. The lungs, together with the aortic segment, were immersed in cold Perfadex and kept in cold storage at 8°C for 4 h.

Preservation of the lungs (V)

Ventricular fibrillation was induced electrically. The animals were then randomized into two different groups. In group one, called the non-ventilation group, the animal was left untouched for 2 h at room temperature. In group two, the ventilator was reconnected after 15 min, and the lungs were ventilated with an FiO₂ of 0.5, a frequency of 15 breaths/min, a minute ventilation of 4 L/min, and no PEEP for 2 h. Apart from the ventilation, the animals were left untouched during the 2-h period at room temperature. After 2 h and 15 min after the declaration of death, a median sternotomy was performed. The pulmonary artery was cannulated via the right ventricle with a 28 F cannula secured with a purse-string suture placed in the outflow tract of the a. pulmonalis. The right and left pleurae were filled with ice slush to cool the lungs. The lungs were perfused antegradely with 2 L of cold Perfadex containing 1.0 mL isotonic trometamol (Addex-THAM, 3.3 mmol/mL; Fresenius Kabi AB, Uppsala, Sweden), 2 mL calcium chloride (0.45 mmol/mL), and 3 mL nitroglycerine (5 mg/mL; BMM Pharma AB, Stockholm, Sweden) at a low perfusion pressure (< 20 mmHg). The lungs were harvested en bloc in a standard fashion, and weighed. A segment (~8 cm) of the

descending aorta was also excised. The lungs, together with the aortic segment, were then immersed in cold Perfadex and kept in cold storage at 8°C for 2 h.

Preservation of the lungs (VI)

Ventricular fibrillation was induced electrically. The tracheal tube was disconnected from the ventilator when circulatory arrest was confirmed. A thermometer was inserted in the pulmonary graft. The animals were randomized into two groups: cold ischemia and warm ischemia. In the cold ischemia group, the pleuras were initially filled with ice slush, whereas in the warm ischemic group, the animals were left untouched. In both groups, the ischemic time was set to 1 h. The temperature probe was inserted such that the tip was in the mid-portion of the lungs (as centrally as possible). The temperatures of the pulmonary grafts were followed continuously during this time. A median sternotomy was then performed and the pulmonary artery was cut; the eventual thrombotic material was collected and measured. The pulmonary grafts were harvested en bloc. The pulmonary arterial branches were studied macroscopically for thrombotic material in both groups; the magnification was 3.5 and the arteries were opened as far as possible distally.

***Ex vivo* lung perfusion (II, III, IV, V)**

EVLP was performed using the extracorporeal perfusion circuit by Medtronic (Medtronic BV; Ex Vivo Lung Evaluation Set). The setup is shown in Figure 1. The system was primed with albumin (500 mL 50 g/L, and 200 mL 200 g/L; Baxter Medical, Kista, Sweden) and 2 units of autologous blood, previously drawn from each donor. Imipenem (0.5 g; Tienam; Merck Sharp & Dohme), insulin (20 IU; Actrapid; Novo Nordisk), and heparin (10,000 IU; LEO Pharma) were added and isotonic trometamol was used to buffer the mixed solution to a temperature-adjusted pH of 7.4. Gas was supplied to the Affinity® membrane oxygenator (Medtronic, Minneapolis, NJ, USA): first, oxygen and CO₂ during the reconditioning phase and then 93% nitrogen and 7% CO₂ during the testing phase, creating a normal venous blood gas in the perfusate to the pulmonary artery (i.e. the oxygenator was used to deoxygenate the perfusate). Before starting perfusion, the pulmonary artery was extended using the excised segment of the descending aorta to facilitate cannulation. The pulmonary artery cannula was then connected to the corresponding tube of the extracorporeal circuit, the air was removed, and the shunt of the circuit was clamped. An endotracheal tube was secured in the trachea with a cotton band and connected to the ventilator. The remnant of the left atrium was left open,

preventing obstruction of the pulmonary outflow, since the perfusion solution flowed directly out into the lung reconditioning box and the left atrium pressure was therefore 0 mmHg. Low-flow perfusion at 25°C was initiated through the lungs. They were gradually warmed by increasing the temperature of the perfusate. When the temperature had reached 32°C, ventilation was started with an inspired oxygen fraction of 0.5 and a minute volume of 1 L/min, with no PEEP. The pump flow was gradually increased, but the pulmonary artery pressure was not allowed to exceed 20 mmHg. With each 1°C increase in temperature, ventilation was increased by a minute volume of 1 L. Normothermia was reached after 20–30 min and PEEP was added to fully expand the lungs and eliminate atelectasis. Blood gases were analyzed during perfusion and full ventilation (Figure 2). The lungs were then disconnected from the EVLP equipment. A collapse test was performed for the final evaluation of the lungs by disconnecting the lungs from the ventilator at the end of inspiration.



Figure 1. The EVLP system with a lung connected to the EVLP system and to the ventilator (study IV).

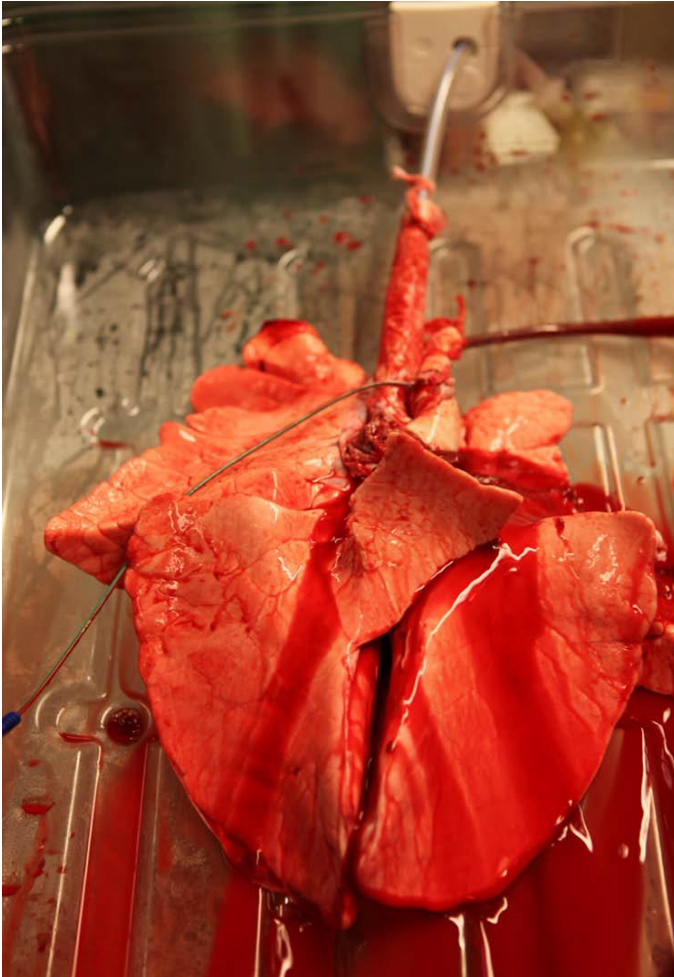


Figure 2. The lung is connected to the EVLP system and to the ventilator (study IV).

Statistics

In study I, we did not do any statistics. We reported each patient separately regarding lung evaluation, transplantation, and outcome.

In studies II–VI, calculations and statistical analyses were performed using GraphPad 4.0 software.

In study II, statistical analysis was performed using one-way ANOVA and Bonferroni's multiple comparison test, comparing all groups. A level of $p < 0.05$ was considered statistically significant, and $p \geq 0.05$ was not considered to be significant (n.s.). The results were presented as median and range or mean and standard error of the mean (SEM).

In study III, statistical analysis was performed using Wilcoxon's test. Any p-value of < 0.05 was considered to be statistically significant, and $p \geq 0.05$ was not considered significant (n.s.). The results were presented as median and range or mean and SEM.

In study IV, statistical analysis was performed using a repeated-measurements general linear model to test the main effect of extended EVLP and subject on pulmonary graft function and quality or pulmonary graft weight. Post hoc testing of the treatment was performed using Dunnett's test with a control (baseline/60 min or weight after organ harvesting). Any p-value of < 0.05 was considered statistically significant, and $p \geq 0.05$ was not considered to be significant (n.s.). The results were presented as mean and SEM.

In study V, statistical analysis was performed using one-way ANOVA and Bonferroni's multiple comparison test, comparing all groups. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***) and $p \geq 0.05$ (not significant, n.s.). The results were presented as mean and SEM.

In the last study (VI), statistical analysis was performed using Mann-Whitney U-test, comparing the two groups. Any p-value of < 0.05 was considered statistically significant, and $p \geq 0.05$ was not considered significant (n.s.). The results were presented as median and range or mean and SEM.

Results

Clinical study, initially rejected donors (I)

During the time period of this study, we did eleven lung transplantations (seven double and four single) with donor lungs that met acceptable standards of quality, and also six transplantations with primarily rejected but reconditioned lungs. Thus, 35% of the lung transplantations were done with reconditioned lungs. The essential characteristics of the donors of the six successfully reconditioned lungs are presented in Tables 1 and 2. Before retrieval, the median (range) PaO₂ at an FiO₂ of 1.0 was 21.1 kPa (11.5–28.7 kPa) (Table 1). After reconditioning, the lungs from these donors produced a median PaO₂ at an FiO₂ of 1.0 of 68.7 kPa (51.6–79.5) (Table 3). The settings of the extracorporeal circulation system and ventilation together with the variables of the physiology achieved are presented in Table 3. The mean total time between organ retrieval and transplantation for the lung transplanted first was 16 h and 43 min, and it was 18:04 for the second (Table 4). The median reconditioning time was 1:29 (range 1:06 to 2:01) (Table 4). The four patients who survived to the 12-month control showed increased 6-minute walking test values compared to the 3-month control, and forced expiratory volume of air in 1 second increased in three patients and was the same in one patient (Table 5). Double sequential lung transplantations were performed without any major surgical complications. In the first five cases, the right lung was transplanted first; in the sixth case, the left lung was transplanted first. The five recipients who survived for 6 months had a postoperative course that was essentially no different from that in patients transplanted with primary accepted donor lungs during the same time period. Recipient no. 6 was a man who had good lung function initially, but on postoperative day 2, he developed sepsis. Blood culture showed the same bacteria as in an inflamed tooth. He never recovered, but died after 95 days because of multiple organ failure. Recipient 4 was a woman who was doing well at the 6-month control, but she developed side effects of cyclosporine, which necessitated a change in immunosuppressive treatment. She died 9 months after the transplantation, due to organ rejection. The surviving four recipients were alive and well without any signs of bronchiolitis obliterans syndrome when approaching the 24-month control at the end of October 2008.

Table 1. The Donors

Variable	Donor:						Median (range)
	1	2	3	4	5	6	
Sex	Female	Male	Female	Female	Female	Male	
Age (years)	63	58	62	52	34	60	59 (34–63)
Height (cm)	158	169	170	173	165	180	170 (158–180)
Weight (kg)	63	94	75	77	55	80	76 (55–94)
Blood group	A+	O+	A+	O+	O+	O+	
CMV	+	+	+	+	—	+	
Cause of death	Cerebral bleeding	Cerebral bleeding	Cerebral bleeding	Cardiac arrest ^a / ROSC	Cardiac arrest ^b / ROSC	Cerebral bleeding	
Smoking	10–15 cig/day 1 year stop	—	40 cig/day	—	15 cig/day	—	
Pao ₂ (kPa) ^c before harvesting (Fio ₂ = 1.0)	28.7	18.2	19.7	11.5	22.4	22.8	21.1 (11.5–28.7)

^a After cerebral bleeding. ^b After epileptic fit. ^c 1kPa = 7.5 mm Hg.

CMV = cytomegalovirus; Fio₂ = fraction of inspired oxygen; Pao₂ = partial pressure of oxygen in arterial blood; ROSC = return of spontaneous circulation (after successful cardiopulmonary resuscitation).

Table 2. The Recipients

Variable	Recipient:						Median (range)
	1	2	3	4	5	6	
Sex	Female	Male	Male	Female	Female	Male	
Age (years)	54	53	55	64	35	63	54 (35–64)
Height (cm)	167.5	182	182	153.5	163	178.5	173 (153.5–182)
Weight (kg)	85.9	90	69.9	55.5	41.5	83.4	76.7 (41.5–90)
Blood group	A–	O+	A–	O–	O+	O+	
CMV	+	–	+	+	+	+	
Diagnosis	COPD	Lung fibrosis	α ₁ -antitryp. deficiency	COPD	Cystic fibrosis	COPD	
Vital capacity (L/% of predicted)	2.5/69	2.3/43	2.6/49	1.3/48	1.7/45	2.8/56	2.4 (1.3–2.8)/49 (43–69)
FEV ₁ (L/% of predicted)	1.2/44	2.0/51	0.7/18	0.5/26	0.9/28	0.9/26	0.9 (0.5–2.0)/27 (18–51)

CMV = cytomegalovirus; COPD = chronic obstructive pulmonary disease; FEV₁ = forced expiratory volume of air in 1 second.

Table 3. Lung Reconditioning

Variable	Donor:						Median (range)
	1	2	3	4	5	6	
Pao ₂ (kPa) ^a after reconditioning.							
Fio ₂ = 1.0	68.7	79.5	68.6	52.3	76.1	51.6	68.7 (51.6–79.5)
Fio ₂ = 0.5	37.4	38.6	19.8	22.0	40.6	31.6	34.5 (19.8–40.6)
Fio ₂ = 0.21	18.5	16.8	15.5	11.4	20.0	11.8	16.2 (11.4–20.0)
Paco ₂ (kPa) (Fio ₂ = 1.0)	4.51	3.32	4.10	3.68	4.47	4.40	4.25 (3.32–4.51)
Ventilation (L/min)	4.0	7.5	7.5	7.0	6.0	6.0	6.5 (4.0–7.5)
Respiratory frequency (min ⁻¹)	15	12	12	16	16	17	16 (12–17)
Perfusion flow (L/min)	2.9	2.8	2.5	3.3	3.2	4.0	3.1 (2.5–4.0)
Hematocrit in Steen solution (%)	10	16	9	10	9	12	10 (9–16)
Pulmonary atrial pressure (mm Hg)	13	17	16	19	12	15	16 (12–19)
Left atrial pressure (mm Hg)	0	0	0	0	0	0	0
Pulmonary vascular resistance (dynes × s × cm ⁻⁵)	358	485	511	467	300	300	413 (300–511)

^a 1kPa = 7.5 mm Hg.

Fio₂ = fraction of inspired oxygen; Paco₂ = partial pressure of carbon dioxide in the arterial blood; Pao₂ = partial pressure of oxygen in arterial blood.

Table 4. Times (Hours:Minutes) for the Different Procedures

Variable	Donor:						Median (range)
	1	2	3	4	5	6	
Cold storage in Perfadex	5:15	9:28	6:38	7:20	6:50	6:13	6:44 (5:15–9:58)
Reconditioning	1:06	1:29	1:24	1:55	1:10	2:01	1:29 (1:06–2:01)
Topical ECMO							
First transplanted lung	4:36	6:18	5:21	5:28	8:36	9:26	5:53 (4:36–9:26)
Second transplanted lung	6:38	10:18	8:06	7:28	11:28	13:34	8:46 (6:38–13:34)
Harvesting to transplantation							
First transplanted lung	10:56	17:45	13:23	16:50	16:36	17:18	16:43 (10:56–17:45)
Second transplanted lung	12:58	21:45	16:08	18:50	19:28	21:26	18:04 (12:58–21:45)

ECMO = extracorporeal membrane oxygenation.

Table 5. Six-Minute Walking Test and Spirometry After Transplantation

Variable	Recipient:						Median (range)
	1	2	3	4	5	6	
6-minute walking test (meter/% of predicted):							
3 months	329/65	330/50	410/64	371/75	476/51	—	371 (329–476)/64 (50–75)
6 months	468/94	359/55	578/93	323/66	565/78	—	468 (323–578)/78 (55–94)
12 months	378/83	380/59	546/89	—	576/82	—	463 (378–579)/83 (59–89)
Vital capacity (L/% of predicted):							
3 months	2.8/76	2.3/43	3.9/75	1.8/69	1.6/42	—	2.3 (1.6–3.9)/69 (42–76)
6 months	3.0/83	2.4/44	3.9/74	2.2/83	1.7/45	—	2.4 (1.7–3.9)/74 (44–83)
12 months	3.0/83	2.8/52	4.1/79	—	2.1/54	—	2.9 (2.1–4.1)/68 (52–83)
FEV ₁ (L/% of predicted):							
3 months	2.1/76	1.8/47	2.6/69	1.6/80	1.5/46	—	1.8 (1.5–2.6)/69 (46–80)
6 months	2.1/78	2.0/51	2.6/69	1.8/80	1.6/50	—	2.0 (1.6–2.6)/69 (50–80)
12 months	2.2/80	2.2/58	2.6/69	—	2.1/64	—	2.2 (2.1–2.6)/67 (58–80)

FEV₁ = forced expiratory volume of air in 1 second.

Experimental studies

Modified versus conventional EVLP (II)

No significant differences were observed in animal weight in the two groups (72 ± 1 kg in the modified EVLP group and 73 ± 2 kg in the conventional EVLP group) ($p > 0.05$). Nor were there any differences in arterial oxygen partial pressure at an FiO_2 of 1.0 (64.8 ± 6.0 kPa in the modified EVLP group and 67.7 ± 1.8 kPa in the conventional EVLP group). No anatomical anomalies, signs of infection, or malignancy were found in any of the animals at autopsy.

Pulmonary graft function

Arterial and venous blood gases

The arterial blood gases and venous blood gases after 5 min of ventilation at an FiO_2 of 1.0 are presented in Table 1. Notice the significant increase in arterial blood gases in both groups after performing one of the maneuvers for eliminating the lung atelectasis. In the modified EVLP group, the PaO_2 was 18.5 ± 7.0 kPa before the maneuver and 64.5 ± 6.0 kPa after the maneuver ($p < 0.001$). In the conventional EVLP group, the PaO_2 was 16.8 ± 3.1 kPa before the maneuver and 46.8 ± 2.7 kPa after the maneuver ($p < 0.01$). Comparing the two groups, the modified EVLP group showed significantly improved PaO_2 after the maneuver compared to the conventional EVLP group ($p < 0.01$) (Figure 1). The airway pressure (AP) is also presented in Table 1. Note the significant decrease in AP in the modified EVLP group before (27.0 ± 2.9 cm H_2O) and after (20.0 ± 2.0 cm H_2O) the maneuver ($p < 0.05$). Note also the increase in AP in the conventional EVLP group before (24.3 ± 1.5 cm H_2O) and after (30.7 ± 1.8 cm H_2O) the maneuver ($p < 0.01$). Comparing the two groups, the modified EVLP group showed lower AP after the maneuver than in the conventional EVLP group ($p < 0.001$) (Figure 2).

Pulmonary vascular resistance

Pulmonary vascular resistance (PVR) was calculated using the formula: $\text{PVR} (\text{dyne} \cdot \text{s}/\text{cm}^5) = [(80 * \text{PAP}) - \text{LAP}] / \text{CO}$, where PAP is the mean pulmonary artery pressure, LAP is the left atrium pressure, and CO is the cardiac output (CO is equivalent to pulmonary artery flow in the EVLP method). The pulmonary vascular resistance was calculated after ventilation with FiO_2 at 1.0 and is also presented in Table 1. In the modified EVLP group, the PVR was

359 ± 57 before the maneuver and 333 ± 48 after the maneuver, a slight but statistically insignificant decrease ($p > 0.05$). In the conventional EVLP group, the PVR was 366 ± 24 before the maneuver and 464 ± 39 after the maneuver ($p < 0.05$). Comparing the two groups, the modified EVLP group showed significantly lower PVR after the maneuver than in the conventional EVLP group ($p < 0.05$) (Figure 3)

Pulmonary graft weight

The lungs were weighed after harvesting and after EVLP to assess the degree of lung edema. The results are shown in Figure 1. Interestingly, the pulmonary grafts receiving the modified EVLP showed unchanged weight before and after the maneuver, indicating a dry pulmonary graft of good quality, while the pulmonary grafts from the conventional EVLP showed increased pulmonary graft weight—indicating a wet, heavy pulmonary graft of poor quality. In the modified EVLP group, the pulmonary graft weight was 723 ± 43 g before the maneuver and 746 ± 43 g after the maneuver (n.s.). In the conventional EVLP group, the pulmonary graft weight was 690 ± 15 g before the maneuver and 973 ± 60 g after the maneuver ($p < 0.001$). Comparing the two groups, the modified EVLP group showed significantly lower pulmonary graft weight after the maneuver than in the conventional EVLP group ($p < 0.001$) (Figure 4).

Hemodynamic data

Pulmonary artery flow (L/min), i.e. cardiac output (CO) in the ex vivo model and pulmonary artery pressure (PAP), were measured continuously. The pulmonary artery flow was not allowed to exceed 4 L/min, and the PAP was not allowed to exceed 20 mmHg. No significant differences in pulmonary artery flow or PAP were seen before and after the different maneuvers in the two groups, or between the two groups during the evaluation of the pulmonary graft. The data are presented in Table 1.

Macroscopic appearance

After completing the lung evaluation, the pulmonary arterial branches were studied macroscopically for thrombotic material by opening the arteries as far distally as possible (Figure 2). No thrombotic material was observed in either of the groups.

Table 1.

	Before & After modified EVLP			Before & After conventional EVLP			Total
	Before	After	p-Value	Before	After	p-value	
FiO₂ 1.0							
PAP (mmHg)	17 ± 2.1	16.7 ± 2.4	n.s.	18 ± 1.2	20 ± 0.0	n.s.	n.s.
CO (L/min)	3.8 ± 0.2	4.0 ± 0.0	n.s.	3.9 ± 0.1	3.5 ± 0.3	n.s.	n.s.
AP (cmH₂O)	27 ± 2.9	20 ± 2.0	< 0.05	24.3 ± 1.5	30.7 ± 1.8	< 0.01	< 0.001
PvCO₂ (kPa)	4.2 ± 0.3	4.4 ± 0.2	n.s.	4.0 ± 0.2	4.0 ± 0.1	n.s.	n.s.
PvO₂ (kPa)	7.5 ± 0.4	8.0 ± 0.5	n.s.	7.6 ± 0.7	7.5 ± 0.3	n.s.	n.s.
PaCO₂ (kPa)	3.9 ± 0.1	3.7 ± 0.1	n.s.	4.0 ± 0.1	3.8 ± 0.1	n.s.	n.s.
PaO₂ (kPa)	18.5 ± 7	64.5 ± 6	< 0.001	16.8 ± 3.1	46.8 ± 2.7	< 0.01	< 0.01
PVR ((dynes · s)/cm⁵)	359 ± 57	333 ± 48	n.s.	366 ± 24	464 ± 39	< 0.05	< 0.05
Weight (g)	723 ± 43	746 ± 43	n.s.	690 ± 15	973 ± 60	< 0.001	< 0.001

FiO₂ = inspired oxygen fraction, PAP = pulmonary arterial pressure, CO = cardiac output / pulmonary artery flow, AP = mean airway pressure, PvCO₂ = venous carbon dioxide partial pressure, PvO₂ = venous oxygen partial pressure, PaCO₂ = arterial carbon dioxide partial pressure, PaO₂ = arterial oxygen partial pressure, PVR = pulmonary vascular resistance.

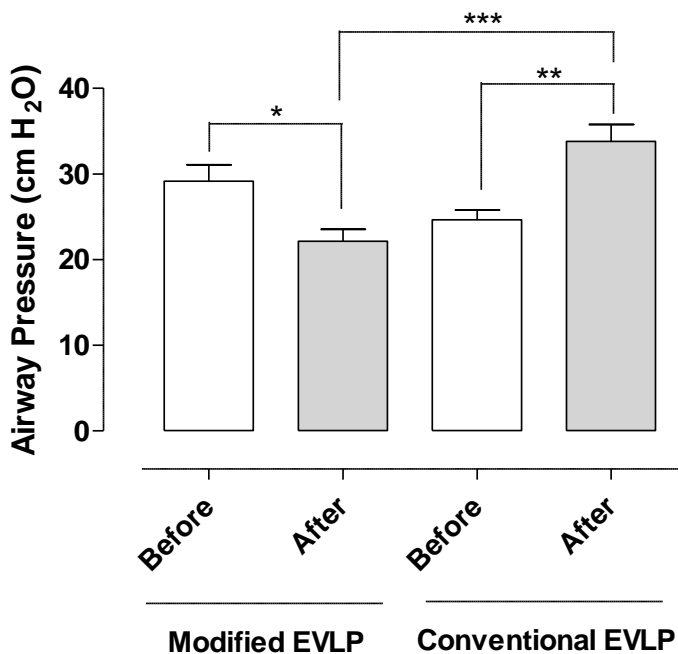


Figure 1

Mean airway pressure (\pm SEM) before and after modified or conventional EVLP in respect of elimination of lung atelectasis after initial EVLP, where the lung is warmed up to 37°C and ventilated according to the gold standard for EVLP. Statistical analysis was done using ANOVA. n = 6 in each group. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

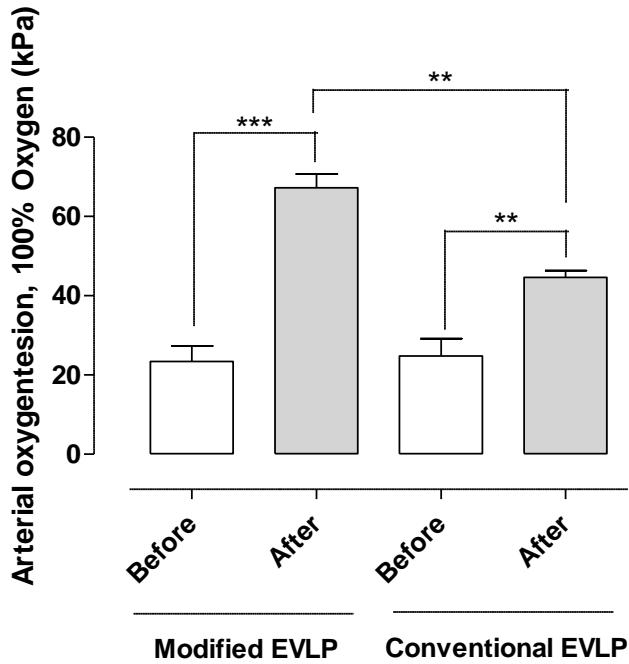


Figure 2

Mean arterial oxygen tension (\pm SEM), before and after modified or conventional EVLP in respect of elimination of lung atelectasis after initial EVLP, where the lung is warmed up to 37°C and ventilated according to the gold standard for EVLP. Statistical analysis was done using ANOVA. n = 6 in each group. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

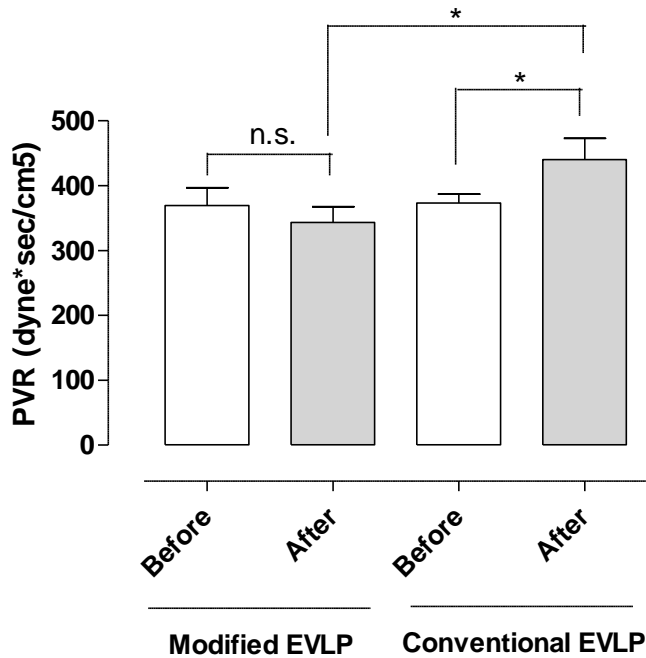


Figure 3

Pulmonary vascular resistance (PVR) (\pm SEM) before and after modified or conventional EVLP in respect of elimination of lung atelectasis after initial EVLP, where the lung is warmed up to 37°C and ventilated according to the gold standard for EVLP. Statistical analysis was performed using ANOVA. N = 6 in each group. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.s., not significant.

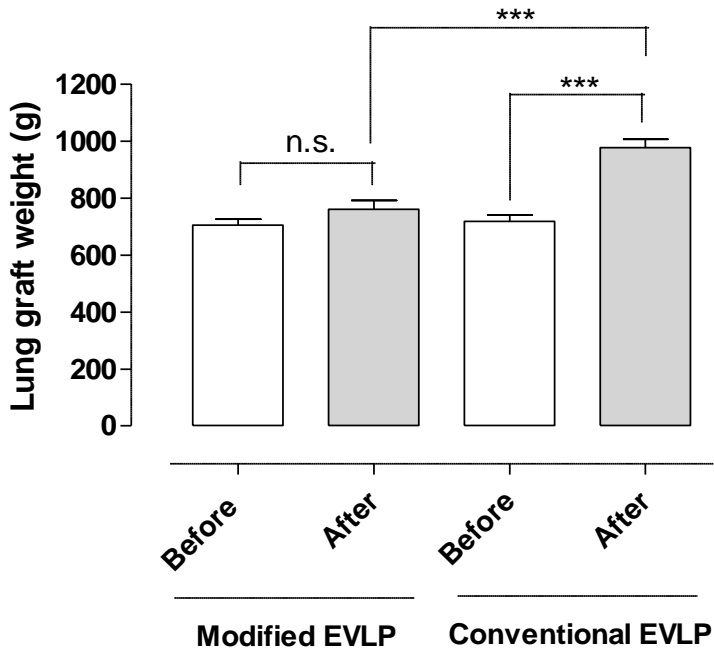


Figure 4

Mean lung weight (\pm SEM), before and after modified or conventional EVLP in respect of elimination of lung atelectasis after initial EVLP, where the lung is warmed up to 37°C and ventilated according to the gold standard for EVLP. Statistical analysis was performed using ANOVA. N = 6 in each group. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.s., not significant.

Heparin and pulmonary graft function (III)

Study groups

No significant differences were observed in animal weight in the two groups (61 ± 2 kg in the HBD non-heparin group and 60 ± 1.5 kg in the HBD heparin group ($p > 0.05$)). Nor were there any differences in PaO_2 at an FiO_2 of 1.0 (62.4 ± 3.4 in the non-heparin group and 66.1 ± 4.4 in the heparin group). No anatomical anomalies, signs of infection, or malignancy were found in any of the animals at autopsy.

Pulmonary graft function

Arterial and venous blood gases

The arterial blood gases and venous blood gases at the different FiO_2 values are presented in Table 1. No significant differences were observed between the two groups.

Pulmonary vascular resistance

The pulmonary vascular resistance was calculated at the different FiO_2 values and is also presented in Table 1. Again, no significant difference was seen between the two groups.

Pulmonary graft compliance

After evaluation of the lungs, they were disconnected from the ventilator and a collapse test was performed. If the lungs do not collapse, this may indicate lung injury, lung edema, or pneumonia. All the lungs from both study groups collapsed as they should, showing good compliance. No significant difference was seen between the lungs in the two groups.

Pulmonary graft weight

The lungs were weighed after harvesting, before EVLP and after EVLP, to assess the degree of lung edema. The results are shown in Figure 2. After harvesting, the mean lung weight in the heparin group was 524 ± 20.5 g, and it was 518 ± 9.9 g in the non-heparin group ($p = 0.99$). After completion of the

lung evaluation, the mean lung weight was 509 ± 23.1 g in the heparin group and 526 ± 9.4 g ($p = 0.31$) in the non-heparin group. Thus, no significant differences were found between the two groups.

Hemodynamic data

No significant differences in pulmonary artery flow or PAP were seen between the two groups during the evaluation of the pulmonary graft at the different fractions of inspired oxygen. The data are presented in Table 1.

Macroscopic appearance

After completion of the lung evaluation, the pulmonary arterial branches were studied macroscopically for thrombotic material by opening the arteries as far distally as possible (Figure 3). No thrombotic material was observed in either of the groups.

Table 1. The *ex vivo* lung perfusion data expressed as median (range) number of heart-beating donors (HBDs) receiving heparin and those without heparin for inspired oxygen fractions of 1.0, 0.5, and 0.21. Statistical analysis was done using Wilcoxon's test to compare the two groups of HBDs with and without heparin regarding lung function. Significance was defined as $p < 0.05$.

	HBDs with Heparin		HBDs without Heparin		p-value
	Median	Range	Median	Range	
<i>FiO₂ 1.0</i>					
PAP (mmHg)	20	(14–20)	20	(16–20)	0.99
Pulmonary artery flow (L/min)	3.0	(2.6–4.0)	3.0	(2.1–4.0)	0.71
PaO₂ (kPa)	69.2	(46.1–77.0)	61.6	(47.9–71.4)	0.44
PaCO₂ (kPa)	3.7	(2.6–3.8)	3.1	(2.9–3.9)	0.99
PvO₂ (kPa)	6.8	(6.2–7.0)	8.7	(5.3–16.1)	0.09
PvCO₂ (kPa)	3.9	(3.8–4.0)	3.5	(2.8–3.8)	0.03
PVR ((dyne · s)/ cm⁵)	543	(280–615)	533	(320–762)	0.99
<i>FiO₂ 0.5</i>					
PAP (mmHg)	20	(15–20)	20	(16–20)	0.99
Pulmonary artery flow (L/min)	3.0	(2.6–4.0)	3.0	(2.1–4.0)	0.71
PaO₂ (kPa)	40.5	(28.1–44.0)	32.8	(25.2–41.7)	0.22
PaCO₂ (kPa)	3.6	(2.4–3.8)	3.3	(2.9–5.1)	0.99
PvO₂ (kPa)	6.6	(6.2–7.0)	8.1	(6.2–14.2)	0.06
PvCO₂ (kPa)	3.8	(2.9–4.1)	3.5	(3.1–3.8)	0.17
PVR ((dyne · s)/ cm⁵)	543	(300–615)	533	(320–762)	0.63

<i>FiO₂ 0.21</i>					
PAP (mmHg)	20	(14–20)	20	(16–20)	0.99
Pulmonary artery flow (L/min)	3.0	(2.6–4.0)	3.0	(2.1–4.0)	0.71
PaO₂ (kPa)	10.5	(9.2–12.1)	12.0	(9.9–15.2)	0.16
PaCO₂ (kPa)	3.4	(2.7–3.7)	3.4	(3.0–4.9)	0.75
PvO₂ (kPa)	6.5	(3.8–6.8)	3.8	(3.5–4.4)	0.06
PvCO₂ (kPa)	3.5	(3.1–3.9)	3.4	(3.2–4.5)	0.75
PVR ((dyne · s)/ cm⁵)	543	(280–615)	533	(320–762)	0.99

FiO₂ = inspired oxygen fraction, PAP = pulmonary arterial pressure, PaO₂ = arterial oxygen partial pressure, PaCO₂ = arterial carbon dioxide partial pressure, PvO₂ = venous oxygen partial pressure, PvCO₂ = venous carbon dioxide partial pressure, PVR = pulmonary vascular resistance.

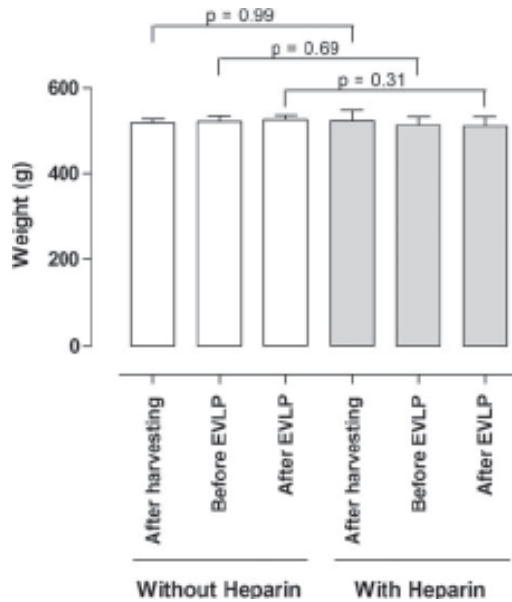


Figure 2.

Mean lung weight (SEM) after harvesting, before EVLP, and after completion of EVLP for pulmonary grafts from the two groups of pigs: one not treated with heparin, and the other treated with heparin. Statistical analysis was performed using Wilcoxon ' s test. Significance was defined as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p > 0.05$ (not significant, n.s.).

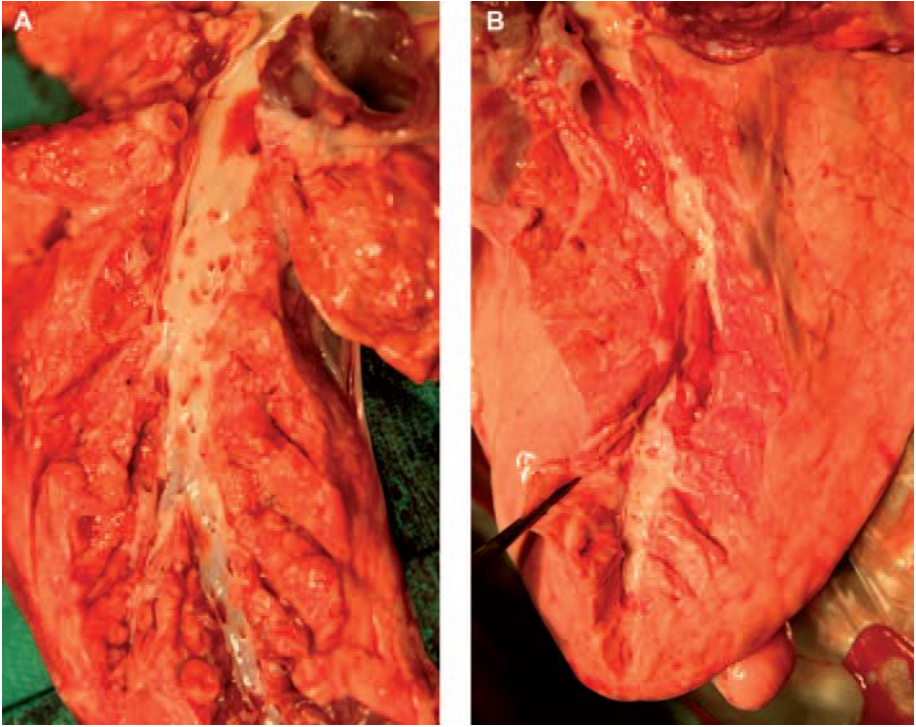


Figure 3. After lung evaluation, the pulmonary arterial branches were studied macroscopically for thrombotic material by opening the arteries as far distally as possible. No thrombotic material was observed in either of the groups. A. A pulmonary graft not treated with heparin. B. A pulmonary graft treated with heparin.

Extended EVLP and pulmonary graft function (IV)

Study groups

The mean weight of the six animals was 59 ± 1.2 kg. The mean partial pressure of oxygen in the blood at an FiO_2 of 1.0 prior to lung harvesting was 61.8 ± 3.2 kPa. No anatomical anomalies, signs of infection, or malignancy were found in the animals at autopsy.

Pulmonary graft function

Arterial and venous blood gases

There was no significant difference in venous and arterial blood gases over time. The value of the pO_2 was 60.8 ± 4.8 kPa after 60 min of EVLP, 64.7 ± 3.3 kPa after 90 min, 67.2 ± 1.9 kPa after 120 min, and 67.1 ± 2.2 kPa after 150 min ($p = 0.48$) (Figure 3A). The value of the venous blood gas (pCO_2) was 3.0 ± 0.2 kPa after 60 min of EVLP, 3.1 ± 0.1 kPa after 90 min, 3.1 ± 0.2 kPa after 120 min, and 3.1 ± 0.1 kPa after 150 min ($p = 0.94$) (Figure 3A).

Pulmonary vascular resistance

Pulmonary vascular resistance (PVR) was calculated using the formula: $\text{PVR} (\text{dyne} \cdot \text{s}/\text{cm}^5) = [(80 * \text{PAP}) - \text{LAP}] / \text{CO}$, where PAP is the mean pulmonary artery pressure, LAP is the left atrium pressure, and CO is the cardiac output. The PVR was 453 ± 78 $\text{dyne} \cdot \text{s}/\text{cm}^5$ after 60, 90, 120, and 150 min of EVLP ($p = 1.0$) (Figure 3C).

Pulmonary graft compliance

After EVLP, the double lungs were disconnected from the ventilator and a collapse test was performed. If the lungs do not collapse, this may indicate injury, edema, or pneumonia. Good compliance was found in all the lungs.

Pulmonary graft weight

The double lungs from the six pigs were weighed after lung harvesting, before EVPL and after EVPL, to assess the degree of lung edema. The mean weight of the pulmonary grafts after harvesting was 574 ± 20 g, it was 541 ± 24 g before EVLP, and it was 668 ± 33 after 150 minutes of EVLP ($p = 0.011$). The results are shown in Figure 4.

Hemodynamic data

The pulmonary artery flow (L/min), i.e. the cardiac output (CO) and the pulmonary artery pressure PAP, was measured continuously. The pulmonary artery flow was not allowed to exceed 4 L/min, and/or the pulmonary artery pressure was not allowed to exceed 20 mmHg. The PAP was 17.8 ± 1.0 mmHg after 60, 90, 120, and 150 min of EVLP ($p = 1.0$) (Figure 3B). The pulmonary artery flow was 3.5 ± 0.4 L/min after 60, 90, 120, and 150 min of EVLP ($p = 1.0$) (Figure 3D).

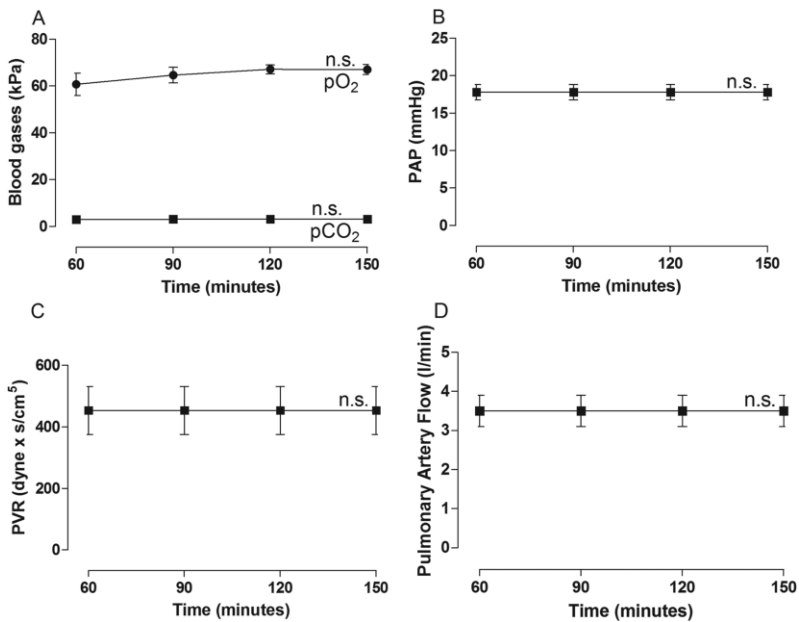


Figure 3. Blood gases (A), pulmonary arterial pressure (PAP) (B), pulmonary vascular resistance (PVR) (C), and pulmonary arterial flow (D) after 60, 90, 120, and 150 min of EVLP. Data shown are the mean \pm SEM of each parameter ($n = 6$). Statistical calculations were performed on data collected at 60, 90, 120, and 150 min using a repeated-measurements general linear model to test the main effect of extended EVLP. Any p -value of < 0.05 was considered statistically significant, and $p > 0.05$ was not considered significant (n.s.).

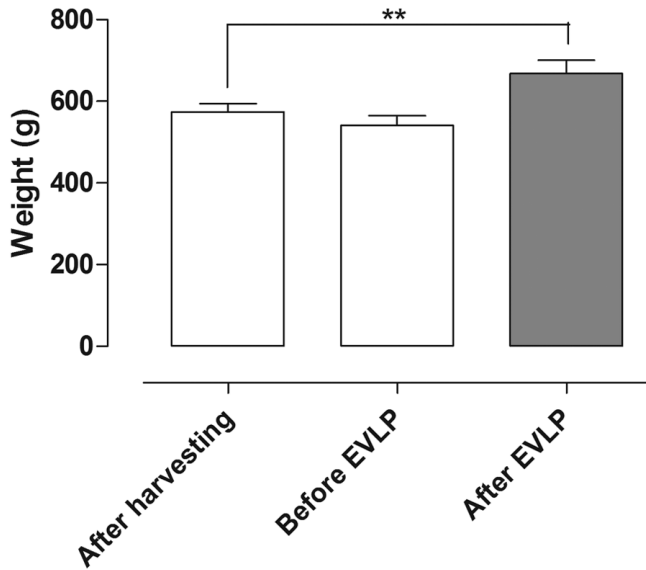


Figure 4. Lung weight after harvesting, before starting EVLP, and after an extended period of EVLP. The results shown are the mean \pm SEM of six experiments. Statistical analysis was done using a repeated-measurements general linear model to test the main effect of extended EVLP and subject on pulmonary graft weight. Any p-value of $p < 0.05$ was considered statistically significant, and $p > 0.05$ was not considered significant (n.s.). Note the significant increase in pulmonary graft weight after the extended period of EVLP.

Ventilation *in situ* (V)

Study groups

No significant differences in animal weight were observed between the two groups (68 ± 1 kg in the non-ventilated group and 72 ± 3 kg in the ventilated group, $p > 0.05$). No anatomical anomalies, signs of infection, or malignancy were found in any of the animals at autopsy.

Elimination of lung atelectasis

In the ventilated group, the PaO_2 increased from 37.1 ± 2.2 kPa to 71.2 ± 2.7 kPa at an FiO_2 of 1.0 after the maneuver ($p < 0.001$), from 13.8 ± 2.0 kPa to 30.0 ± 0.8 kPa at an FiO_2 of 0.5 after the maneuver ($p < 0.001$), and 9.2 ± 0.7 kPa to 13.9 ± 0.7 kPa at an FiO_2 of 0.21 after the maneuver ($p < 0.001$). The PVR and airway pressure were also significantly reduced after the maneuver. The results are shown in Table 1 and in Figure 1.

In the non-ventilated group, all the lungs showed fulminant lung edema, and three out of six lungs were hepatized in the lower lobes bilaterally. PAP, pulmonary artery flow, blood gases, PVR, and airway pressure were not significantly changed after the modified EVLP procedure.

Arterial and venous blood gases

The arterial blood gases and venous blood gases after 5 min of ventilation at an FiO_2 of 1.0, 0.5, and 0.21 are presented in Table 2.

At an FiO_2 of 1.0, the PaO_2 was 71.2 ± 2.7 kPa in the ventilated group and 9.6 ± 0.8 kPa in the non-ventilated group ($p < 0.001$). At an FiO_2 of 0.5, the PaO_2 was 30.0 ± 0.8 kPa in the ventilated group and 7.6 ± 0.3 kPa in the non-ventilated group ($p < 0.001$). At an FiO_2 of 0.21, the PaO_2 was 13.9 ± 0.7 kPa in the ventilated group and 6.2 ± 0.2 kPa in the non-ventilated group ($p < 0.001$) (Figure 2).

Airway pressure

The mean airway pressure (AP) is also presented in Table 2. In the ventilation group, the AP was 19.7 ± 1.9 cmH₂O and in the non-ventilated group it was 34.4 ± 2.8 cmH₂O ($p < 0.001$) at an FiO_2 of 1.0, 0.5, and 0.21.

Pulmonary graft function

Pulmonary vascular resistance

Pulmonary vascular resistance (PVR) was calculated using the formula: $PVR \text{ (dyne} \cdot \text{s/cm}^5) = [(80 * PAP) - LAP] / CO$, where PAP is the mean pulmonary artery pressure, LAP is the left atrium pressure, and CO is the cardiac output (CO is equivalent to pulmonary artery flow in the EVPL method).

The pulmonary vascular resistance was calculated after ventilation at an FiO_2 of 1.0, 0.5, and 0.21 and it is presented in Table 2. In the ventilation group, the PVR was $373 \pm 13 \text{ dyne} \cdot \text{s/cm}^5$, and in the non-ventilated group the PVR was $383 \pm 8 \text{ dyne} \cdot \text{s/cm}^5$ ($p > 0.05$) at an FiO_2 of 1.0, 0.5, and 0.21.

Pulmonary graft weight

In the non-ventilated group, the pulmonary graft weight was $650 \pm 33 \text{ g}$ before the EVLP and $1,167 \pm 69 \text{ g}$ after the EVLP ($p < 0.001$). In the ventilated group, the pulmonary graft weight was $687 \pm 9 \text{ g}$ before the EVLP and $707 \pm 12 \text{ g}$ after the EVLP (n.s). Comparing the two groups, the ventilated group showed significantly lower pulmonary graft weight after the EVLP than the non-ventilated group ($p < 0.001$) (Figure 3).

Hemodynamic data

Pulmonary artery flow (L/min), i.e. cardiac output (CO) in the *ex vivo* model and pulmonary artery pressure (PAP), were measured continuously. The pulmonary artery flow was not allowed to exceed 4 L/min, and the PAP was not allowed to exceed 20 mmHg. Interestingly, no significant differences in pulmonary artery flow or PAP were seen in the two groups. The data are presented in Table 2.

Macroscopic appearance

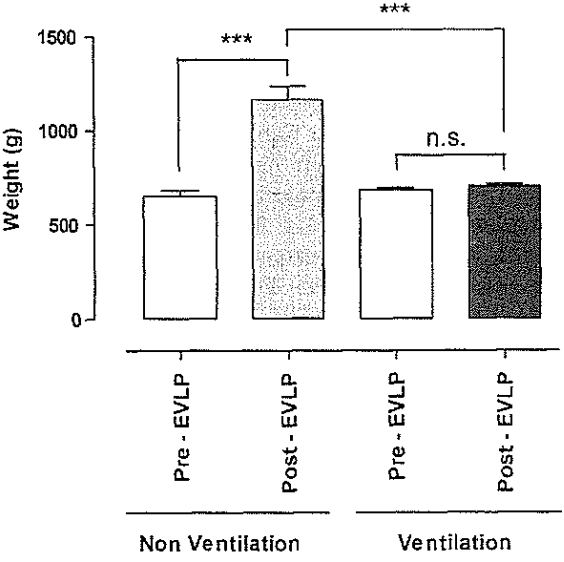
No thrombotic material was observed in either of the groups. A fulminant lung edema was present in all the lungs in the non-ventilated group. Three out of six lungs were hepatized in the lower lobes bilaterally.

Table 1. Ischemia Two Hours

	No Ventilation		Ventilation	
		Mean \pm SEM	Mean \pm SEM	p-value
FiO ₂ = 1.0	PAP	19.2 \pm 0.4	18.7 \pm 0.7	> 0.05
	Flow	4.0 \pm 0.0	4.0 \pm 0.0	> 0.05
	PvCO ₂	4.3 \pm 0.1	4.4 \pm 0.2	> 0.05
	PvO ₂	6.8 \pm 0.2	7.7 \pm 0.7	< 0.05
	PaCO ₂	3.9 \pm 0.1	3.7 \pm 0.1	> 0.05
	PaO ₂	9.6 \pm 0.8	71.2 \pm 2.7	< 0.001
	PVR	383 \pm 8	373 \pm 13	> 0.05
	AP	34.4 \pm 2.8	19.7 \pm 1.9	< 0.001
	FiO ₂ = 0.5	PAP	19.2 \pm 0.4	18.7 \pm 0.7
Flow		4.0 \pm 0.0	4.0 \pm 0.0	> 0.05
PvCO ₂		4.3 \pm 0.2	3.6 \pm 0.3	> 0.05
PvO ₂		6.1 \pm 0.1	6.8 \pm 0.2	> 0.05
PaCO ₂		4.0 \pm 0.1	3.5 \pm 0.2	> 0.05
PaO ₂		7.6 \pm 0.3	30.0 \pm 0.8	< 0.001
PVR		383 \pm 8	373 \pm 13	> 0.05
AP		34.4 \pm 2.8	19.7 \pm 1.9	< 0.001
FiO ₂ = 0.21		PAP	19.2 \pm 0.4	18.7 \pm 0.7
	Flow	4.0 \pm 0.0	4.0 \pm 0.0	> 0.05
	PvCO ₂	4.5 \pm 0.1	3.8 \pm 0.1	> 0.05
	PvO ₂	6.0 \pm 0.1	6.6 \pm 0.2	> 0.05
	PaCO ₂	4.0 \pm 0.2	3.4 \pm 0.1	> 0.05
	PaO ₂	6.2 \pm 0.2	13.9 \pm 0.7	< 0.001
	PVR	383 \pm 8	373 \pm 13	> 0.05
	AP	34.4 \pm 2.8	19.7 \pm 1.9	< 0.001

FiO₂ = inspired oxygen fraction, PAP = pulmonary arterial pressure, CO = cardiac output / pulmonary artery flow, AP = mean airway pressure, PvCO₂ = venous carbon dioxide partial pressure, PvO₂ = venous oxygen partial pressure, PaCO₂ = arterial carbon dioxide partial pressure, PaO₂ = arterial oxygen partial pressure, PVR = pulmonary vascular resistance.

Figure 1. Mean lung weight (\pm SEM) after EVLP of lungs exposed to 2 hours of warm ischemia. The ventilated group received ventilation of the lungs during the warm ischemic time while the non-ventilated group did not. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.s., not significant.



Warm versus cold ischemia and pulmonary graft thrombosis (VI)

Study groups

No significant differences in animal weight were observed in the two groups (58.5 ± 0.4 kg in the warm ischemic group and 58.2 ± 0.4 kg in the cold ischemic group) ($p > 0.05$). Nor were there any differences in arterial oxygen partial pressure at an inspired oxygen fraction of 1.0 (63.4 ± 0.9 kPa in the warm ischemic group and 61.3 ± 1.0 kPa in the cold ischemic group) before introduction of ventricular fibrillation. No anatomical anomalies, signs of infection, or malignancy were found in any of the animals at autopsy.

Pulmonary graft temperature

The temperature of the pulmonary graft was followed continuously during the ischemic time of 1 hour in both groups. At time 0 min, the temperature was 37.1 ± 0.1 degrees in the warm ischemic group and 37.0 ± 0.1 in the cold ischemic group ($p > 0.05$). After 15 min, the temperature was 37.0 ± 0.1 in the warm ischemic group and 24.6 ± 0.2 in the cold ischemic group ($p < 0.001$). After 30 min, it was 36.8 ± 0.1 in the warm ischemic group and 21.3 ± 0.2 in the cold ischemic group ($p < 0.001$). After 45 min, the temperature was 36.7 ± 0.1 in the warm ischemic group and 18.1 ± 0.1 in the cold ischemic group ($p < 0.001$), and after 60 min the temperature was 36.6 ± 0.1 in the warm ischemic group and 14.6 ± 0.1 in the cold ischemic group ($p < 0.001$) (Figure 2).

Thrombotic material

After one hour of either warm or cold ischemia, the pulmonary artery was opened and any thrombotic material was collected and measured. In the warm ischemic group, no thrombotic material could be found in the pulmonary artery (0.0 ± 0.0 mL), and in the cold ischemic group 6.8 ± 0.2 mL thrombotic material was found in the pulmonary artery ($p < 0.001$). The pulmonary grafts were then harvested en bloc and the pulmonary arterial branches were studied macroscopically for thrombotic material by opening the arteries as far distally as possible (Figure 1). Any thrombotic material was collected and measured. In the warm ischemic group, no thrombotic material could be found in the pulmonary arterial branches, and in the cold ischemic group, 2.3 ± 0.1 mL thrombotic material was found in the branches of the pulmonary artery ($p < 0.001$) (Figure 3).

Figure 1

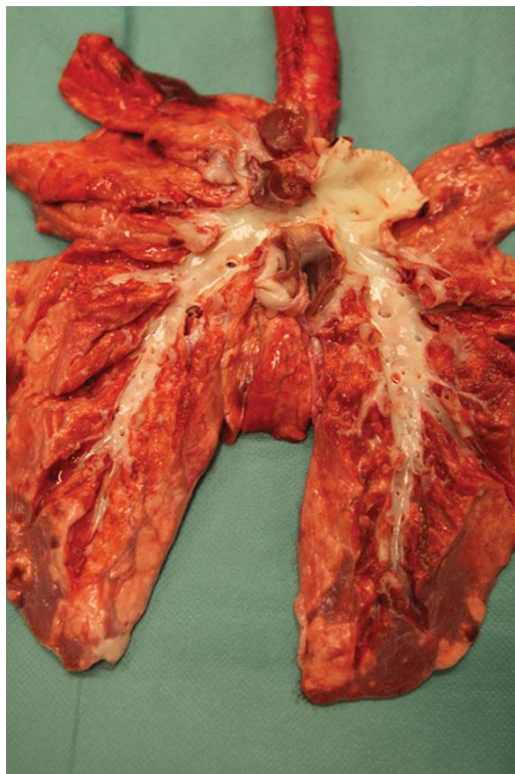


Figure 1. The branches of the pulmonary artery were studied macroscopically for thrombotic material by opening the arteries as far as possible distally.

Figure 2

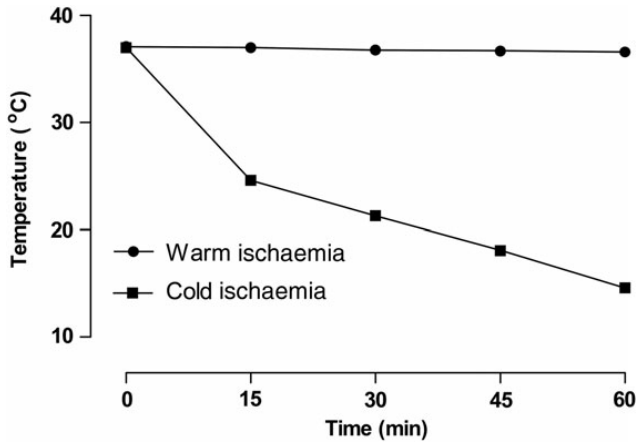
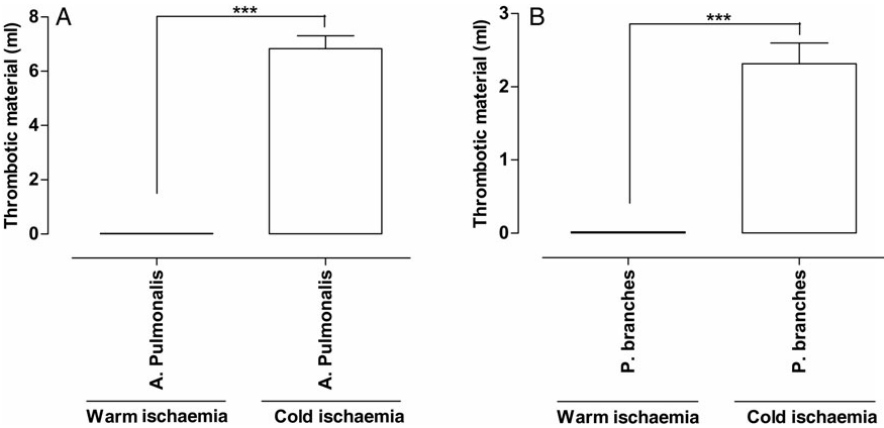


Figure 2. The temperature in the pulmonary grafts was continuously followed in both groups. Note how the temperature fell post mortem in the cold ischemic group, while the temperature was unchanged in the warm ischemic group. The results are presented as mean \pm SEM. Significance was defined as $p < 0.05$. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. n.s., not significant.

Figure 3. After one hour of either warm or cold ischemia, the pulmonary artery was opened and thrombotic material was collected and measured in the pulmonary arteries (A) and in the more distal pulmonary branches (B). Note the absence of thrombotic material in the warm ischemic group. The results are presented as mean \pm SEM. Significance was defined as $p < 0.05$. *** $p < 0.001$.



Comments

Lack of suitable organs continues to be a major problem in lung transplantation. Only 15–25% of lungs from donors of at least one other organ are transplanted, being the lowest graft acceptance rate of any solid organ. As the number of patients referred for lung transplantation exceeds the number of donor organs available, it is evident that donor identification and appropriate assessment of the suitability of a potential lung donor should be optimized in order to maximize lung donation rates.

A new method for *ex vivo* lung perfusion and evaluation has been developed by Steen and colleagues, which was used successfully for the first time in humans on donor lungs from an uncontrolled DCD donor at Lund University Hospital, Sweden, in 2001. Further progress has been made since then, and the method is now used for the reconditioning of marginal and unacceptable donor lungs from DBD donors. This method has the potential to considerably increase the number of lungs available for transplantation. In this thesis, I have described the first six double lung transplantations to be performed with DBD donor lungs that were reconditioned *ex vivo*, which had been rejected for transplantation by the Scandiatransplant, Eurotransplant, and UK transplant organizations because of insufficient arterial oxygen tension. The three-month survival was 100%, and four patients were alive and well without any signs of bronchiolitis obliterans syndrome two years after transplantation. If we were to use a large proportion of the DBD marginal donors available, the number of patients transplanted would increase but we would not get unlimited access to organs. Even after reconditioning, many of these marginal donors will be marginal donors with increased mortality and prolonged intensive care time as a result.

It has been estimated that the EU has some 375,000 potential DCD donors every year. The initial strategy for those patients has been heparinization followed by bilateral pleural drainage and topical cooling of the lungs—then followed by lung harvesting, reconditioning, and evaluation of lung function according to Steen’s method. This has been performed, but ethical and legal issues have arisen. It has been considered unethical to administer heparin and give heart massage to distribute it in the body, and to put in pleural drainage and cool the lungs after resuscitation has been stopped. This must be performed before the families are informed and we do not know the donor’s opinion regarding organ donation. In many countries this is not legal. Furthermore, many doctors do not like to be a doctor and try to save a patient’s life—and

then suddenly swift and give heparin and insert pleural drainage in order to preserve the organs, and later on harvest and evaluate them for transplantation. Ethically, many countries have tried to come around this by legalizing donation preparation work such as administration of heparin. Despite this, the method is beset with ethical issues. Lately, organ harvest followed by lung transplantation has been performed using another strategy. When the controlled DCD donor's circulation has stopped, there is a 2- to 5-min "hands off" period and then the lungs are harvested and flush perfused, followed by EVLP evaluation and eventually transplantation. The transplanted patients have done well, but there have been both ethical and legal issues. We are not convinced that this is the way to go, and have created our own algorithm for DCD donors. This algorithm give us more time and allows us to use donor lungs from both controlled and uncontrolled DCD donors.

It is well known that if you only administrate heparin they can tolerate a maximum of 1 hour of warm ischemia [30]. Reconditioning and evaluation of these lungs can be performed, with satisfactory results. However, 1 hour is considered to be too short a time to perform declaration of death, inform the relatives, take all the necessary blood samples, transport the patient to an operating theater, and discuss with cardiac surgeons. All these factors have meant that the method has not yet had a breakthrough.

In the present thesis, there are two findings that have enabled us to use a new algorithm for DCD donation of lungs. The finding that as long as the lungs are at body temperature (38°C), there will be no thrombus formation in the grafts (paper VI) is a very important finding. There is no requirement for heparin to be given during the first hour after death, since the lungs will have a temperature of 38°C one hour after circulatory death. We know from earlier that the lung can tolerate one hour of warm ischemia. A DCD donor can then be left untouched for one hour without warm ischemic damage, and without thrombus formation in the graft [31, 32]. However, one hour is a short period of time. We can perform declaration of death after 30 min, followed by checking of the donation registry and communication with the next of kin. After doing this, the hour will have been used up but all the legal and ethical issues will have been solved properly. The second important finding in this thesis is the fact that ventilation of the DCD donor (paper V), starting 1 hour after circulatory death, preserves the lung function in an excellent way for at least 2.5 to 3 hours. This finding gives us enough time to perform all the necessary preparations for harvesting of the lungs (taking all the necessary blood samples, transportation of the patient to an operating theater, discussion

with cardiac surgeons, and so on) without any decrease in graft function. These two findings combined are of utmost importance for creation of an algorithm that makes it possible to use DCD donor lungs in an ethically and legally correct way—at the same time as preserving good lung function. Then we can perform *ex vivo* reconditioning and evaluation just to ensure that the donor lungs are in good condition. They will have been exposed to both warm ischemia and the risk of thrombus formation, and it is important to investigate their function before any decision to do a transplant. This might expand the potential donor pool substantially, resulting in an increased transplantation frequency.

We have also investigated the effect of extended EVLP perfusion after evaluation is finished (paper IV). The extended evaluation did not further improve pulmonary graft function. Furthermore, we found a way to eliminate atelectasis without harming the lungs during the EVLP procedure. The normal way to get rid of atelectasis is to increase PEEP. However, this cannot be performed without the risk of harming the lungs. To solve this we switch off perfusion of the lungs and increase PEEP. There will then be much more room for the atelectatic area of the lung to expand, since there is no competition with fluid regarding space. It is of utmost importance to get rid of atelectasis if one is to be able to evaluate the true lung function in a proper way.

We have searched and received ethical permission to determine whether we can reproduce these observations using human lungs. Furthermore, the ethical committee actually considers it unethical to avoid transplantation if these lungs fulfill the criteria for transplantation after EVLP evaluation.

Conclusions

Our clinical results show that lungs from marginal DBD donors can be successfully transplanted after undergoing an EVLP procedure.

Severe atelectasis may be eliminated using the EVLP technique.

Administration of pre-harvest heparin appears to be of no importance for good lung graft preservation.

Extended EVLP treatment did not give further improvement in pulmonary graft function.

Ventilation *in situ* appears to protect the pulmonary graft from the negative effects of warm ischemia in the DCD situation.

Warm ischemia rather than cold ischemia appears to protect the pulmonary graft from thrombosis in uncontrolled non-heparinized DCD donors.

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and lastly,

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Papers I–VI