TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

In situ lung perfusion is a valuable tool to assess lungs from donation after circulatory death donors category I–II

Caroline Van De Wauwer,[1] Anita J. Munneke,[2] Gerwin E. Engels,[2] Foke M. Berga,[1] Gerhard Rakhorst,[2] Maarten W. Nijsten,[3] Massimo A. Mariani[1] and Michiel E. Erasmus[1]

1 Department of Cardiothoracic Surgery, University Medical Center Groningen, Groningen, The Netherlands
2 Department of Biomedical Engineering, University Medical Center Groningen, Groningen, The Netherlands
3 Department of Critical Care, University Medical Center Groningen, Groningen, The Netherlands

Keywords
category I–II, donation after circulatory death, ISLP, lactate, lung transplantation.

Correspondence
C. Van De Wauwer, Department of Cardiothoracic Surgery, University Medical Centre Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands.
Tel.: 0031503616161;
fax: 0031503611347;
e-mail: c.van.de.wauwer@umcg.nl

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 29 September 2012
Revision requested: 1 November 2012
Accepted: 6 January 2013
Published online: 6 February 2013

doi:10.1111/tri.12068

Summary

Donations after circulatory death (DCD) lung grafts are an alternative to extend the donor pool in lung transplantation. This study investigates the use of an in situ lung perfusion system (ISLP) in the donor to evaluate category I–II lungs. Pigs were sacrificed by ventricular fibrillation. All animals underwent 20 min of cardiopulmonary resuscitation and 5 min hands-off period after which heparin was administered. In group [WI-1], this was followed by 1 h of warm ischemia (WI) and 2 h of topical cooling (TC). In group [WI-2], 2 h of WI was followed by 1 h of TC. In group [WI-0], there was a minimal period of WI and no TC. In all three groups, the lungs were then evaluated during 60 min with ISLP. [WI-0] lungs showed a significantly higher compliance and Δ PO_2/FiO_2 compared with [WI-1] and [WI-2]. $PaCO_2$ and lactate production were higher in [WI-2] versus [WI-0]. Wet/Dry weight ratio was significantly higher in [WI-2] compared with [WI-0] in two lung biopsy locations. A high W/D weight ratio was correlated with a lower compliance, higher lactate production, and a higher $PaCO_2$. ISLP is an effective way to assess the quality of lungs from category I–II DCD donors.

Introduction

Lung transplantation is a lifesaving treatment for well-selected patients with benign end-stage pulmonary disease. One of the major limitations of this treatment is the donor organ shortage. Only 15% of lungs from multi-organ donors are available for transplantation [1]. The use of lungs from donation after circulatory death (DCD) donors is one of the strategies to increase the donor pool. However, evaluating the lung function in uncontrolled DCD remains challenging.

Normothermic *ex vivo* lung perfusion (EVLP) was developed as a tool for assessing lung viability and for reconditioning of marginal and unacceptable donor lungs [2]. Recently, Cypel *et al.* reported in a prospective study that extended normothermic EVLP allows an assessment of DCD donor lungs and donation after brain death (DBD) donor lungs. Transplantation of these lungs led to results comparable with conventionally selected lungs [3]. The group of Varella initially evaluated uncontrolled DCD donor lungs using a pulmonary flush technique [4]. Recently, lungs were assessed using EVLP before implantation [5] .

Donation after circulatory death is inevitable associated with a warm ischemia (WI) period. Warm ischemia is the ischemia of cells and tissues under normothermic conditions. It leads to endothelial dysfunction and to alveolar type II cell dysfunction resulting in pulmonary edema and graft dysfunction during reperfusion [6–9]. There is experimental and clinical evidence that 1 h of WI does not compromise the function of the pulmonary graft [10]. However, reperfusion after 2 h of WI results in deterioration of the pulmonary graft [6].

No study so far has evaluated the feasibility of an evaluation of lungs from uncontrolled DCD donors with a lung - perfusion system in the donor. In in situ lung perfusion (ISLP), the lungs remain in the deceased body. The heart –lung block is connected to a reperfusion system and a bed site assessment is performed. At that time, there is no extra manipulation of the lungs by means of flush or harvest.

The primary endpoint of this study was to investigate the feasibility of an ISLP to make an assessment of the donor lung quality. The secondary endpoint was to determine predictors for lung injury in this model.

Material and methods

Experimental groups

Twelve domestic pigs ($n = 4$/group) (average weight: 47.5 kg) were randomly divided into three equal groups. In all groups, the animals were sacrificed by inducing ventricular fibrillation. This was followed by cardiopulmonary resuscitation for 20 min during which the systolic blood pressure was above 50 mmHg. During cardiopulmonary resuscitation, the animals were ventilated with an inspiratory oxygen fraction (FiO_2) of 0.5, a tidal volume of 5 ml/kg, and a respiratory rate of 5 breaths/min. After a 5-min hands-off period, during which the endotracheal tube was disconnected from the ventilator and left open to the air, heparin (15 000 IU intravenously) was given. The heparin was circulated by performing closed chest massage (20 times) and restarting of the ventilation. In group [WI-1], this was followed by 1 h of warm ischemia (WI), 2 h of topical cooling (TC), and 1 h of ISLP. In group [WI-2], the warm ischemic period was extended up to 2 h followed by 1 h of TC and 1 h of ISLP. Finally, in group [WI-0], ISLP was performed after a short period of WI, without TC.

Animal preparation

Animals were premedicated, positioned in a prone position, and then intubated with an endotracheal tube. After re-positioning in supine position, the animals were ventilated with a FiO_2 of 0.5, a tidal volume of 10 ml/kg body weight, a respiratory rate of 10–12 breaths/min and a positive end-expiratory pressure of 5 cmH_2O. Anesthesia was maintained with sevoflurane 2–3% and muscle relaxation with continuous infusion of pancuronium bromide (Pavulon 2 mg/ml; Organon, Teknika, Boxtel, The Netherlands). Hemodynamic monitoring via a catheter placed in the right common carotid artery and blood gas analysis were performed in all animals. The right external jugular vein was used for infusion of fluids. Lung function was continuously monitored.

All animals received human care in compliance with the *Principles of Laboratory Animal Care*, formulated by the National Society for 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 (NIH Publication No. 85-23, Revised 1996). The study was approved by the institutional animal care and use committee of the University of Groningen.

The pigs were sacrificed according to the study protocol described above. In [WI-1] and [WI-2], the cadavers were left untouched at room temperature. Temperature of the lung was measured via a probe within the endotracheal tube. At the end of the WI, a sternotomy was performed and the lungs were inspected.

Topical cooling was started with a combination of cold saline and buffered Perfadex®. Endotracheal, chest cavity, and rectal temperature were measured. Target was an endobronchial temperature of 12 °C. At the end of the TC, the pulmonary artery was cannulated through the right ventricular outflow tract and isolated with a ligature around the catheter distal to the pulmonary valve. A small catheter was placed in the pulmonary artery for measurement of pulmonary artery pressure (PAP). The left atrium was cannulated through the apex of the left ventricle with a second cannula and secured with a purse-string. Another small catheter was placed in the left atrium for measurement of the left atrium pressure (LAP).

After confirmation of asystole, the sternotomy was performed in [WI-0]. The ISLP was started after cannulation of the pulmonary artery and left atrium.

In situ lung perfusion

The ISLP evaluation was performed with the use of a Lung Assist. The Lung Assist (Organ Assist BV, Groningen, The Netherlands) is a combined heating/cooling and centrifugal pump system. A flow probe and temperature probes are also included. It is used in combination with a reservoir, an oxygenator, and a ventilator. For the ISLP, the system was primed with 1.5 l of Steen® solution. Steen solution is a buffered extracellular solution containing dextran and human serum albumine with an optimal colloid osmotic pressure (295 ± 20 mOsm/kg) preventing formation of lung edema [11]. The ISLP was performed using an acellular perfusate with a hematocrit <10%.

Technique of ventilation and controlled reperfusion

Reperfusion of the heart–lung block was started, after retrograde flush of the tubing, with oxygenated Steen solution (FiO_2 0.21) at room temperature (20 °C). PAP was gradually increased to a maximum of 15 mmHg and the left atrial pressure was kept at 3–5 mmHg by adjusting the height of the blood reservoir. This resulted in warming up

of the lung and a gradual increase in pulmonary artery flow. This, up to maximum, calculated flow of 40% of the total cardiac output (cardiac output = 100 ml/kg) or a mean PAP of 15 mmHg. When a temperature of 32 °C was reached, ventilation was started with a FiO_2 0.5, a tidal volume of 10 ml/kg, a frequency of 12 breaths/min, and PEEP of 5 cmH_2O. At that moment, the perfusate was continuously deoxygenated to a PaO_2 of 6.6–8 kPa with a gas mixture of CO_2 (8%), O_2 (6%), and N_2 (86%).

Assessment of the graft

The functional graft parameters were recorded up to 1 h. PAP (mmHg) and LAP (mmHg) were measured continuously. A flow probe on the inflow line measured the pulmonary artery flow (PAF) (l/min). Pulmonary vascular resistance (PVR) was calculated using the formula: PVR = [PAP – LAP]/PAF and expressed in Wood units. Dynamic lung compliance (Compl) and plateau airway pressure (Plat AwP) were recorded. PO_2, PCO_2, and lactate of the inflowing and outflowing perfusate were measured at 45 and 60 min. Oxygenation capacity was calculated using the $\Delta PO_2/FiO_2$ ratio (kPa) (ΔPO_2 = perfusate left atrium PO_2–perfusate pulmonary artery PO_2). The lactate production during the last 15 min of reperfusion was calculated (outflow 60 min–outflow 45 min). The transpulmonary lactate gradient (Δ lactate) was calculated as the difference between outflow lactate and inflow lactate at 0, 45, and 60 min.

Temperature (°C) of the inflowing and outflowing perfusate was continuously measured, the last being considered as the graft temperature.

At the end of the reperfusion, biopsies were taken from apical anterior part, the apical posterior part, the basal anterior part, and the basal posterior part of the right lung in [WI-0] and from the left lung in [WI-1] and [WI-2]. W/D weight ratio was assessed as parameter for lung edema.

Histology

At the end of the experiment, tissue samples were obtained from the right lung in the [WI-0] and from the left lung in [WI-1] and [WI-2]. Specimens were fixed in 6% formaldehyde, dehydrated and stained with hematoxylin and eosin, and examined for pathologic changes under a light microscope. Histological analysis was performed by one experienced pathologist who was blinded for the experimental setup.

Correlations

The W/D weight ratios of all three groups were correlated with all parameters to determine predictors for lung injury.

Statistical analysis

Data were analyzed using Graphpad Prism 5 (San Diego, CA, USA). Graft parameters among three study groups were compared using a one-way ANOVA test. Graft parameters between two study groups were compared using an unpaired *t*-test. Data presented in the figures are mean with standard error of the mean (SEM). Data in the tables are presented as mean with standard deviation (SD). A *P*-value <0.05 was considered as significant.

Results

Study groups

There was no significant difference for PaO_2/FiO_2 at 50% and 100% of oxygen, plateau airway pressure, peak airway pressure, and compliance prior to sacrifice in the three groups.

Pulmonary graft function

Dynamic lung compliance

Compliance (ml/mbar) was significantly better in [WI-0] compared with [WI-1] and [WI-2] from 45 min until the end of the reperfusion (at 45 min: 45 ± 5 vs. 30 ± 3 and 15 ± 4; at 60 min: 46 ± 7 vs. 28 ± 3 and 13 ± 4, respectively) ($P < 0.05$) (Fig. 1a). From 45 to 55 min of reperfusion, compliance was also significantly higher in [WI-1] compared with [WI-2] ($P < 0.05$).

Plateau airway pressure

At the end of the reperfusion, the plateau airway pressure (mbar) was lower in [WI-0] and in [WI-1] compared with [WI-2] (18 ± 2 and 17 ± 2 vs. 36 ± 10; respectively) ($P > 0.05$) (Fig. 1b).

Pulmonary vascular resistance

There was no significant difference between the three groups. At the end of the reperfusion, PVR (Wood units) was lower in [WI-0] compared with [WI-1] en [WI-2] (5 ± 1 vs. 7 ± 2 and 10 ± 4) ($P > 0.05$) (Fig. 1c).

Oxygenation capacity

At 45 min of reperfusion, lung oxygenation function ($\Delta PO_2/FiO_2$) (kPa) was significantly better in [WI-0] compared with [WI-2] (53 ± 2 vs. 35 ± 5) ($P = 0.0119$). There was no significant difference between [WI-1] and [WI-2] at 45 min. At 60 min of reperfusion, there was a significant difference between [WI-0] and [WI-1] vs. [WI-2] (52 ± 4 and 44 ± 4 vs. 33 ± 4) ($P = 0.0077$) (Fig. 2a).

$PaCO_2$

There was more CO_2 in the outflowing perfusate in [WI-2] compared with [WI-0] at 45 min and 60 min of

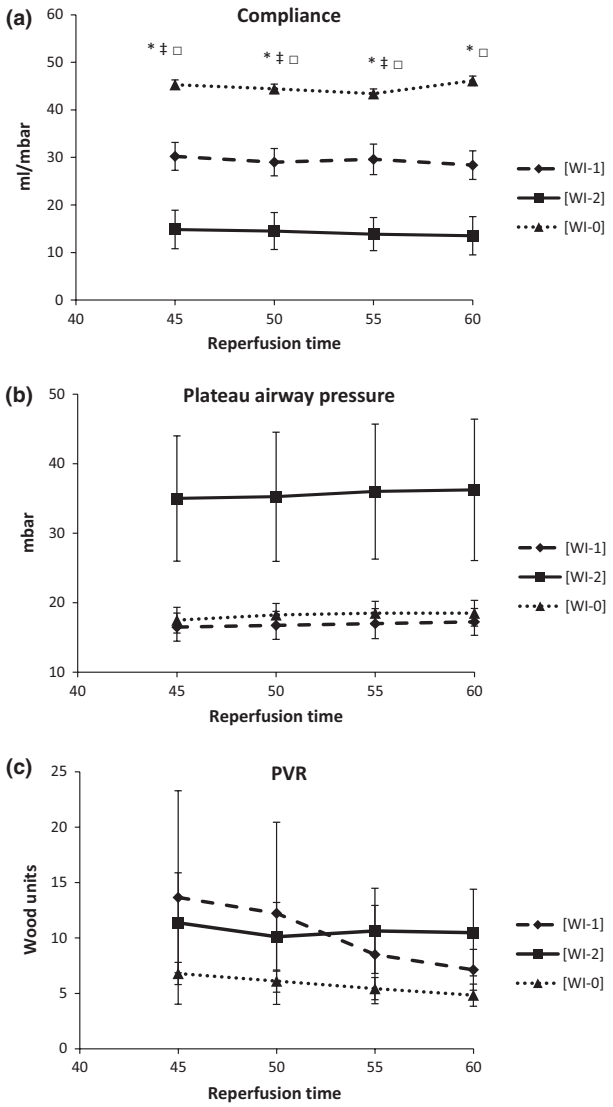

Figure 1 Pulmonary graft function (mean ± SEM) from 45 to 60 min after the start of in situ reperfusion. (a) Dynamic lung compliance: *$P < 0.05$ [WI-2] versus [WI-0], □ $P < 0.05$ [WI-1] versus [WI-0] and ‡$P < 0.05$ [WI-2] versus [WI-1]. (b) Plateau airway pressure: NS between groups. (c) Pulmonary vascular resistance: NS between groups.

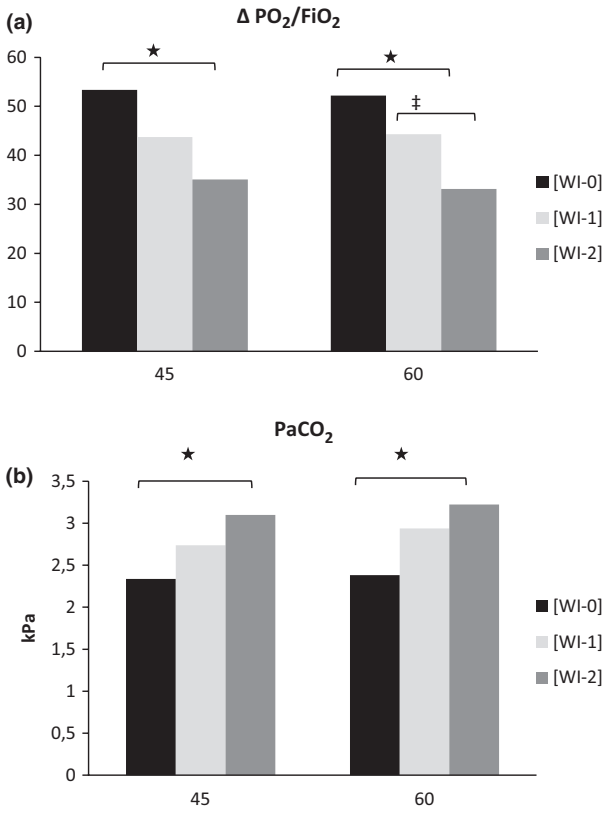

Figure 2 Oxygenation capacity and $PaCO_2$. (a) Δ PO_2/FiO_2: *$P < 0.05$ [WI-2] versus [WI-0] and ‡$P < 0.05$ [WI-2] versus [WI-1]. (b) $PaCO_2$: *$P < 0.05$ [WI-2] versus [WI-0].

reperfusion [3.10 ± 0.15 vs. 2.34 ± 0.10 ($P = 0.0156$) and 3.22 ± 0.15 vs. 2.38 ± 0.05 ($P = 0.0463$), respectively] (Fig. 2b). There was no significant difference in the pCO_2 of the inflowing perfusate.

Lactate

There was a continuous sharp increase in lactate in [WI-2]. In [WI-0] and [WI-1], the sharp increase was followed by a lesser increase in the lactate ($P > 0.05$) (Fig. 3a).

The lactate production (mmol/l) during the last 15 min of reperfusion is higher in [WI-2] compared with [WI-1] and [WI-0] (0.93 ± 0.23 vs. 0.54 ± 0.11 and 0.3 ± 0.11) ($P = 0.0588$) (Fig. 3b).

In [WI-0] and [WI-1], there was an increase in Δ lactate during the first 45 min of reperfusion followed by a decrease during the last 15 min ($P > 0.05$) (Fig. 3c). Instead in [WI-2], the Δ lactate continuously increased during reperfusion.

Wet-to-dry weight ratio

W/D ratio was significantly lower in [WI-0] compared with [WI-1] and [WI-2] in the basal anterior lung biopsy. In the apical anterior lung biopsy, W/D ratio was lower in [WI-0] compared with [WI-2] (Table 1).

Correlations

There was a negative correlation between compliance and the W/D weight ratios in the four biopsies and a positive correlation among $PaCO_2$, lactate production, and the W/D weight ratios in the four biopsies. For plateau airway pressure, there was a positive correlation in three of four biopsies and for Δ lactate in two of four biopsies (Table 2).

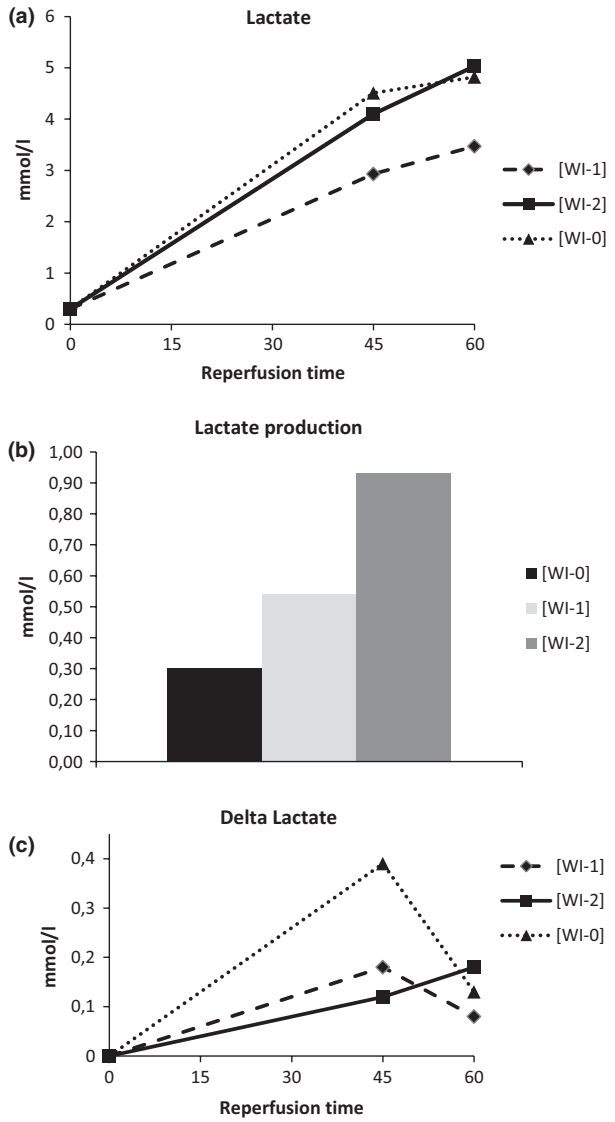

Figure 3 (a) Lactate levels during the reperfusion: NS between groups. (b) Lactate production during the last 15 min of reperfusion: NS between groups. (c) Transpulmonary lactate gradient (Δ lactate): NS between groups.

Gross appearance

There was pulmonary edema with edema fluid in the endotracheal tube in [WI-2]. In [WI-0] and [WI-1], there was no pulmonary edema.

Histology

Microscopically, lung injury was scored assessing alveolar congestion, hemorrhage, vascular thrombosis, infiltration, or aggregation of neutrophils. There were no significant differences among the three groups.

Discussion

Normothermic EVLP has proven to be a successful technique for assessment and reconditioning of marginal and unacceptable donor lungs from DBD donors and DCD donors in clinical and experimental work [11–13]. However, in DCD category I–II, lung function is often unknown at the time of recovery. Therefore, we established an ISLP system that can assess the lungs in the donor without preceding pulmonary flush preservation. The graft function at the end of ISLP in [WI-0] was comparable to the baseline parameters demonstrating that ISLP is a safe way to assess lungs from NHBD category I–II.

A key finding of our study is that resuscitation in combination with WI causes damage even in this ideal experimental setting. Another finding of this study is that pulmonary graft function of one animal in [WI-2] was equivalent to that of the lungs in [WI-1] animal. This observation, however, requires further study. A previous study of Egan *et al.* reports that some animals survived an observation period after 2 h of WI and after 5 h of WI [14]. Also Hayama reports good results after 2 h of WI in a study using dogs [15]. This may suggest that DCD category I–II donor lungs after both 1 and 2 h of WI may be suitable for transplantation after evaluation and subsequent reconditioning with ISLP or EVLP.

Until today, PO_2 is the gold standard for evaluation of donor lungs. However, there is recent evidence that during EVLP and subsequently during ISLP, physiologic parameters are of greater importance than PO_2. Yeung *et al.* demonstrated that *ex vivo* PO_2 may not be the first indication of lung injury. In this study, pulmonary edema was associated with a decrease in compliance and an increase in airway pressure before a decrease in PO_2 [16].

In our study, there was a significant correlation between the compliance and W/D weight ratio. von Neergaard demonstrated that when the lung is completely filled with

Table 1. W/D weight ratio in all three study groups.

	[WI-0]	[WI-1]	[WI-2]	*P*	
Apical anterior	4.55 ± 0.17	5.17 ± 0.13	5.87 ± 0.82	0.0132	[WI-0] versus [WI-2]
Apical posterior	4.92 ± 0.17	5.10 ± 0.15	5.87 ± 0.91	0.0736	
Basal anterior	4.77 ± 0.32	7.02 ± 1.48	7.19 ± 1.37	0.0307	[WI-0] versus [WI-2] [WI-0] versus [WI-1]
Basal posterior	4.78 ± 0.17	6.14 ± 1.55	7.33 ± 1.62	0.0621	

All data are expressed as mean ± SD.

Table 2. Correlations between W/D and other parameters.

		Compliance	pCO_2	Plateau Airway Pressure	Δ Lactate	Lactate production
Apex Anterior	*r*	−0.81	0.74	0.87	0.51	0.65
	P	0.0007	0.003	<0.0001	0.045	0.011
Apex Posterior	*r*	−0.62	0.64	0.91	0.73	0.50
	P	0.016	0.012	<0.0001	0.0034	0.047
Basal Anterior	*r*	−0.69	0.88	0.47	0.063	0.50
	P	0.0065	<0.0001	0.061	0.42	0.049
Basal Posterior	*r*	−0.65	0.94	0.63	0.22	0.56
	P	0.0112	<0.0001	0.013	0.24	0.028

saline, the lung compliance increases. However, in pulmonary edema, not all alveoli are filled with fluid. A recent report shows that in pulmonary edema, the liquid-filled edematous alveoli shrink resulting in an expansion of the air-filled neighbor alveoli. Subsequently, there is an overdistension of the alveoli reducing the compliance of the air-filled alveoli and hence the overall lung compliance [17]. The correlation that we found between compliance and W/D ratio might well be explained by this mechanism.

We also found a relation between the $PaCO_2$ and an increased W/D weight ratio. The gas exchange in the lung is determined by the balance between the capillary flow and the alveolar ventilation. In pulmonary edema, this balance is disturbed causing an intrapulmonary shunt [18]. A decrease in PaO_2 and an increase in $PaCO_2$ is seen when the intrapulmonary shunt rises considerably. Under such conditions, the PaO_2 will be relatively independent of the changes in FiO_2. We hypothesize that in [WI-2] animals, the alveolo-capillary membrane may suffer an injury resulting in macroscopic lung edema and an increased $PaCO_2$.

Also an increased pulmonary lactate production was correlated with an increased W/D ratio indicating injury in our NHBD setting. The higher pulmonary lactate production in [WI-2] was associated with a higher W/D ratio.

In [WI-0] and [WI-1], the Δ lactate initially increased followed by a decrease. However, in the more injured [WI-2] group, there was a continuous increase. As the ISLP circuit is closed with regard to nonvolatile metabolites, the rise of lactate must be the result of lactate production from pulmonary glucose degradation and of lactate release during lung injury [19]. Hypoxia is not necessarily the cause of pulmonary lactate production, as also well-oxygenated lungs are known to be net lactate producers [20]. Moreover, hypoxemia owing to pulmonary insufficiency in clinical studies did not correlate with a rise in lactate level or the lactate-pyruvate ratio (L/P ratio) [21,22]. Moreover, in the acute respiratory distress syndrome and sepsis, with lungs under aerobic conditions, the lungs were a primary contributor of lactate to the circulation [23,24]. Finally, a study on acute lung injury demonstrates the release of lactate by the lung by measuring the gradient over the lung [25].

A recent report evaluating declined lungs from DBD donors demonstrated two distinct patterns in L/P ratio. In some lungs, the L/P ratio increased and then decreased suggesting the anaerobic use of glucose at the start of the EVLP followed by a more physiologic lactate production. In the other group, the L/P ratio remained high reflecting sustained anaerobic metabolism in more injured lungs [26]. This resulted in a higher peak airway pressure in the latter group. In the high L/P group, EVLP was finished after 8 h in one lung as a result of significant pulmonary edema. Our results support their theory.

The Madrid group recently updated their experience with 29 lung transplants from uncontrolled DCD donors and reported a high incidence of primary graft dysfunction (PGD) [4,5]. Our results confirm that the DCD category I–II lungs are already injured at the time of harvesting. The notion that PGD led to higher mortality resulted in a more thorough evaluation of high-risk donors with EVLP after the initial evaluation in the donor. On the basis of our findings, we believe that evaluation and reconditioning by machine lung perfusion may be a very useful procedure. This might be either ISLP or EVLP. A major advantage of ISLP is that it provides a go/no-go at an earlier stage. With the ISLP, there is the possibility to assess the lungs at the time of abdominal organ recovery. A disadvantage is that the lung perfusion system should be transported together with the donor team. When necessary, re-assessment with EVLP can be performed in the recipient hospital.

In 2010, there were 15 DCD donors category I–II in the Netherlands resulting in eight kidney donation procedures. There was no assessment or donation of lungs. In our own hospital in 2010, 8 of 61 patients could be eligible as DCD II lung donors (personal communication). On a nationwide basis, such an extension would considerably enlarge the donor pool.

This study has several limitations. First, the use of a nonprotective ventilation mode might have caused a modest lung injury in [WI-2]. Lung protective ventilation attenuates

alveolar stretch and related injury during mechanical ventilation [27].

Second, the results of a study with a small sample size and with a limited evaluation period need to be confirmed in a model with a longer reperfusion time or a transplant model. Because of the short reperfusion period, there were no histological differences.

Finally, in this study, all animals developed sudden cardiac arrest from myocardial fibrillation. This is different from the clinical situation where exsanguination and myocardial infarction are the most common causes of death in the uncontrolled DCD donors. These patients do not always develop sudden cardiac arrest and the agonal phase and subsequently inflammatory lung injury may be more important.

In conclusion, we demonstrated that ISLP is an effective procedure to make a quality assessment of lungs from DCD category I–II in the donor. In addition, the reported data suggest that compliance, pCO_2 and lactate production may be predictors for lung injury.

Authorship

CVDW, AJM, GEE, MEE: participated in research design, writing of the paper, performance of the research, and data analysis. FMB: participated in research design, performance of the research, and data analysis. GR: participated in research design, performance of the research. MWN, MAM: participated in writing of the article.

Funding

The authors have declared no funding.

Acknowledgements

This study was supported in part by a grant of Vitrolife AB, Göteborg, Sweden and by a grant of Organ Assist BV, Groningen, the Netherlands.

References

1. Hornby K, Ross H, Keshavjee S, Rao V, Shemie SD. Non-utilization of hearts and lungs after consent for donation: a Canadian multicentre study. *Can J Anaesth* 2006; **53**: 831.
2. Cypel M, Keshavjee S. The clinical potential of ex vivo lung perfusion. *Expert Rev Respir Med* 2012; **6**: 27.
3. Cypel M, Keshavjee S. Extracorporeal lung perfusion. *Curr Opin Organ Transplant* 2011; **16**: 469.
4. Rodriguez DA, Del Rio F, Fuentes ME, *et al.* Lung transplantation with uncontrolled non-heart-beating donors. Transplantation. Donor prognostic factor and immediate evolution post transplant. *Arch Bronconeumol* 2011; **47**: 403.
5. Van Raemdonck DE, Jannis NC, De Leyn PR, Flameng WJ, Lerut TE. Warm ischemic tolerance in collapsed pulmonary grafts is limited to 1 hour. *Ann Surg* 1998; **228**: 788.
6. Novick RJ, Gehman KE, Ali IS, Lee J. Lung preservation: the importance of endothelial and alveolar type II cell integrity. *Ann Thorac Surg* 1996; **62**: 302.
7. Inci I, Arni S, Acevedo C, *et al.* Surfactant alterations following donation after cardiac death donor lungs. *Transpl Int* 2011; **24**: 78.
8. Allison RC, Kyle J, Adkins WK, Prasad VR, McCord JM, Taylor AE. Effect of ischemia reperfusion or hypoxia reoxygenation on lung vascular permeability and resistance. *J Appl Physiol* 1990; **69**: 597.
9. Van De Wauwer C, Neyrinck AP, Geudens N, *et al.* Retrograde flush following warm ischemia in the non-heart-beating donor results in superior graft performance at reperfusion. *J Surg Res* 2009; **154**: 118.
10. Moradiellos Diez FJ, Naranjo JM, Cordoba M, *et al.* First successful transplantations after ex vivo evalutation of uncontrolled non-heart-beating donor human lungs. *Interact Cardio Vasc Thorac Surg* 2010; **11**: S18.
11. Cypel M, Yeung JC, Liu M, *et al.* Normothermic ex vivo lung perfusion in clinical lung transplantation. *N Engl J Med* 2011; **364**: 1431.
12. Van Raemdonck D. Thoracic organs: current preservation technology and future prospects; part 1: lung. *Curr Opin Organ Transplant* 2010; **15**: 150.
13. Dark JH. Lung transplantation from the non-heart beating donor. *Transplantation* 2008; **86**: 200.
14. Egan TM, Lambert CJ, Reddick R, Ulicny KS, Keagy BA, Wilcox BR. A strategy to increase the donor pool: use of cadaver lungs for transplantation. *Ann Thorac Surg* 1991; **5**: 1113.
15. Hayama M, Date H, Oto T, Aoe M, Andou A, Shimizu N. Improved lung function by means of retrograde flush in canine lung transplantation with non-heart-beating donors. *J Thorax Cardiovasc Surg* 2003; **125**: 901.
16. Yeung JC, Cypel M, Machuca TN, *et al.* Physiologic assessment of the ex vivo donor lung for transplantation. *J Heart Lung Transplant* 2012; **31**: 1120.
17. Perlman CE, Lederer DJ, Bhattacharya J. Micromechanics of alveolar edema. *Am J Respir Cell Mol Biol* 2011; **44**: 34.
18. D'Alonzo GE, Dantzker DR. Respiratory failure, mechanisms of abnormal gas exchange, and oxygen delivery. *Med Clin North Am* 1983; **67**: 557.
19. Fisher AB. Intermediary metabolism of the lung. *Environ Health Perspect* 1984; **55**: 149.
20. Sanchez PG, Bittle GJ, Burdorf L, Pierson RN III, Griffith BP. State of Art: Clinical ex vivo lung perfusion: Rationale, current status, and future directions. *J Heart Lung Transplant* 2012; **31**: 339.
21. Eldridge F. Blood lactate and pyruvate in pulmonary insufficiency. *N Engl J Med* 1966; **274**: 878.
22. Mitchell AM, Cournand A. The fate of circulating lactic acid in the human lung. *J Clin Invest* 1955; **34**: 471.

23. Brown SD, Clark C, Gutierrez G. Pulmonary lactate release in patients with sepsis and the adult respiratory distress syndrome. *J Crit Care* 1996; **11**: 2.
24. Bellomo R, Kellum JA, Pinsky MR. Transvisceral lactate fluxes during early endotoxemia. *Chest* 1996; **110**: 198.
25. Kellum JA, Kramer DJ, Lee K, Mankad S, Bellomo R, Pinsky MR. Release of lactate by the lung in acute lung injury. *Chest* 1997; **111**: 1301.
26. Koike T, Yeung JC, Cypel M, *et al.* Kinetics of lactate metabolism during acellular normothermic ex vivo lung perfusion. *J Heart Lung Transplant* 2011; **30**: 1312.
27. Seeley EJ, McAuley DF, Eisner M, *et al.* Decreased respiratory system compliance on the sixth day of mechanical ventilation is a predictor of death in patients with established acute lung injury. *Respir Res* 2011; **12**: 52.