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Hearts From DCD Donors Display Acceptable Biventricular Function After Heart Transplantation in Pigs

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Cardiac transplantation is in decline, in contrast to other solid organs where the number of solid organ transplants from donors after circulatory death (DCD) is increasing. Hearts from DCD donors are not currently utilized due to concerns that they may suffer irreversible cardiac injury with resultant poor graft function. Using a large animal model, we tested the hypothesis that hearts from DCD donors would be suitable for transplantation. Donor pigs were subjected to hypoxic cardiac arrest (DCD) followed by 15 min of warm ischemia and resuscitation on cardiopulmonary bypass, or brainstem death (BSD) via intracerebral balloon inflation. Cardiac function was assessed through load-independent measures and magnetic resonance imaging and spectroscopy. After resuscitation, DCD hearts had near normal contractility, although stroke volume was reduced, comparable to BSD hearts. DCD hearts had a significant decline in phosphocreatine and increase in inorganic phosphate during the hypoxic period, with a return to baseline levels after reperfusion. After transplantation, cardiac function was comparable between BSD and DCD groups. Therefore, in a large animal model, the DCD heart maintains viability and recovers function similar to that of the BSD heart and may be suitable for clinical transplantation. Further study is warranted on optimal reperfusion strategies.

Keywords: Donation after circulatory death, heart transplantation

Abbreviations: ATP, adenosine triphosphate; BSD, brainstem death; CO, cardiac output; CPB, cardiopulmonary bypass; CVP, central venous pressure; DCD, donation after circulatory death; EF, ejection fraction; ESPVR, end-systolic pressure–volume relationship; EDPVR, end-diastolic pressure–volume relationship; EDV, end-diastolic volume; ESV, end-systolic volume; Hct, hematocrit; Hb, hemoglobin; LV, left ventricle; MAP, mean arterial pressure; pCr, phosphocreatine; P_i, inorganic phosphate; PRSW, preload recruitable stroke work; RVU, relative volume units; RV, right ventricle; SV, stroke volume.

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Introduction

Procurement of organs after circulatory arrest in the organ donor has increased the provision of organs for transplantation. Renal, liver and lung transplantation have benefited from donation after circulatory death (DCD), with clinical outcomes being comparable to those obtained after transplantation from brainstem dead donors (1–3). The initial era of cardiac transplantation was pioneered using hearts procured after donor cardiac arrest (4,5). Despite this precedent and a substantial body of experimental work suggesting its feasibility (6–12), DCD donors have not been adopted as an alternative donor source for cardiac transplantation in the current era. Organ donation from DCD donors predominantly occurs in "controlled" environments such as the intensive care unit. Similar to brainstem dead donors, DCD donors have often suffered a severe neurologic insult with no prospect for recovery. However, they do not meet criteria required for diagnosis of brainstem death (BSD). Consequently, a decision to abort further supportive therapy is made on clinical grounds and circulatory arrest is awaited. In the interval between discontinuation of support and procurement, organs are exposed to adverse conditions including hypoxia, hypotension and ischemia. Subsequent reperfusion of organs may also result in further injury. Nevertheless, reperfusion of the DCD donor with extracorporeal membrane oxygenation (ECMO), has been proposed as a method to optimize organ function and retrieval (13). As such, our group resuscitated the heart of a DCD donor on cardiopulmonary bypass (CPB; Ref. 14). Although left ventricle (LV) function recovered well, concerns were raised regarding right ventricle (RV) function. Nevertheless, reperfusion of the DCD heart in the donor, on CPB or ECMO, is attractive as it can be accomplished expeditiously, therefore minimizing the warm ischemic time that is inherent to the DCD process (13). We therefore

undertook a comprehensive functional and metabolic assessment of hearts resuscitated after circulatory arrest in a porcine model of DCD donation. Myocardial energetics and changes in high-energy phosphate stores were analyzed during circulatory arrest, reperfusion and resuscitation. Functional assessment in the donor and orthotopic transplantation of the heart were also undertaken to assess the capacity of the DCD heart to support both the donor and recipient circulation.

Methods

The experimental protocol was approved by the Animal Care Committees of the University of Manitoba and the National Research Council of Canada in accordance with guidelines set forth by the Canadian Council on Animal Care and the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 86–23, revised 1996).

Anesthesia, monitoring and baseline measurements

Female domestic pigs were employed as a model of brain death, donation after circulatory death and heart transplantation (mean weight 62 ± 5 kg). Premedication was undertaken with intramuscular injection of midazolam (0.4 mg/kg) and ketamine (20 mg/kg). Anesthesia was maintained using inhalational isoflurane (2.0%). Paralysis was induced using 0.2 mg/kg of pancuronium. Oral endotracheal intubation was established for institution of mechanical ventilation and for general anesthesia. A median sternotomy incision was undertaken, the left internal mammary artery and vein were dissected and cannulated for measurement of arterial blood pressure and central venous pressure respectively. A 5F conductance catheter (Millar Instruments Inc, Houston, TX, USA) was inserted via an arteriotomy in the right common carotid artery and advanced retrogradely into the LV for measurement of LV pressure and volume. Right ventricular pressure–volume data was also collected by introducing the conductance catheter into the RV through a small arteriotomy in the main pulmonary artery. Pressure–volume loops were obtained from both ventricles following occlusion of the inferior vena cava.

Circulatory arrest, establishment of extracorporeal perfusion and cardiac resuscitation

Systemic heparinization was established with 300 units/kg of heparin in preparation for extracorporeal perfusion. A standard CPB circuit comprising of a membrane oxygenator, roller pump and heat exchanger was used for extracorporeal perfusion. Mechanical ventilation was discontinued in the paralyzed animal resulting in asphyxiation and circulatory arrest. Before cessation of mechanical ventilation, an additional dose of ketamine (1 mg/kg) midazolam (0.1 mg/kg) and pancuronium (0.1 mg/kg) was administered, ensuring adequate anesthesia. After the onset of cardiocirculatory arrest, an additional 15-min warm ischemic period was observed before commencement of extracorporeal perfusion. Oxygenated normothermic blood was delivered into the ascending aorta at 5 L/min thereby achieving reperfusion of the donor, including the heart. Ventricular dysrhythmia was treated with internal DC defibrillation (20 J). Hyperkalemia (K^+ > 6 mmol/L) was treated with infusion of insulin-dextrose. Acidosis was corrected with administration of 8.4% sodium bicarbonate. Reperfusion was maintained until satisfactory cardiac function was achieved. After this period, extracorporeal perfusion was weaned to allow the heart to independently support the circulation. Measurement of serum catecholamine levels in venous blood was performed with high-performance liquid chromatography (HPLC).

Brainstem death

A 14F balloon-tipped catheter was introduced into the cranial cavity through a burr-hole. A median sternotomy was performed for insertion of monitoring and conductance catheters. Baseline pressure–volume data was acquired from both ventricles for assessment of contractility and compliance. After this the intracranial balloon catheter was slowly inflated with 30 mL of normal saline. This maneuver consistently resulted in increased intracranial pressure and subsequent compression and herniation of the brainstem. BSD was confirmed through the observation of the classical hyperdynamic response of hypertension and tachycardia, an apnea test, EEG monitoring and magnetic resonance imaging (MRI) of the brain. Measurement of serum catecholamine levels in venous blood was performed with HPLC.

Magnetic resonance spectroscopy

After BSD or DCD heart resuscitation a subgroup of animals (n = 5) underwent MR spectroscopy to assess myocardial energy metabolism. Animals were placed into a 3 Tesla Siemens MR scanner (Sonata, Magnetom, Siemens, Erlangen, Germany). A purpose-built MR surface coil was positioned and secured over the anterior wall of the LV. Transmural ^{31}P spectroscopy was undertaken using ECG gating. Myocardial energy metabolism was assessed through the measurement inorganic phosphate (P_i), phosphocreatinine (pCr) and three peaks of adenosine triphosphate (α, β and γ peaks). Intracellular pH was calculated by analyzing the frequency difference between P_i and pCr peaks.

Cine cardiac MRI

Biventricular chamber volumes and function were evaluated using cine cardiac MRI with a 3-Tesla Siemens MR scanner (Sonata, Magnetom, Siemens, Erlangen, Germany). A four-element phased array coil was used with two coils placed on the anterior chest wall and two placed on the posterior chest wall. Cine images were acquired with ECG trigger during repeated breath-hold to achieve 25 images covering a cardiac cycle. Images were analyzed using the MASS version 4.2 software (MEDIS Medical Imaging Systems, Leiden, Netherlands). Endocardial contour of the ventricular walls were manually traced on all images containing the LV and RV in each end-diastolic and end-systolic slice. Sum of the marked areas was used to calculate the end-diastolic volume (EDV) and the end-systolic volume (ESV). Stroke volume (SV) and ejection fraction (EF) were then calculated.

Pressure–volume measurements

Load independent contractility of the RV and LV was assessed using the ESPVR and the preload recruitable stroke work (PRSW). Diastolic function was assessed using the end-diastolic pressure–volume relationship (EDPVR). Relative volume units (RVU) obtained from conductance measurements were calibrated into actual volumes using measurements of left and right EDV and ESV obtained during cine cardiac MRI. Pressure–volume data was also used to measure load-dependent hemodynamic measures: cardiac output (CO), stroke volume (SV), end-diastolic (EDV) and end-systolic (ESV) volumes, arterial elastance (E_a), dP/dt max and dP/dt min.

Orthotopic heart transplantation

After hemodynamic measurements of the resuscitated DCD and BSD donor heart were concluded, 1 L of cold (4°C) crystalloid cardioplegia solution containing 100 mg of lidocaine was administered into the isolated aortic root to arrest the heart. Topical cold saline (4°C) was also used to supplement myocardial cooling. The donor heart was excised and cold storage was achieved through submersion of the organ in cold saline (4°C). Concurrently, the recipient animal was anesthetized and mechanically ventilated and surgically prepared as described earlier. The donor heart was removed from cold storage and implanted with a standard biatrial anastomotic technique in sequence: left atrium, pulmonary artery and ascending aorta. Before removal of the aortic cross clamp an additional 500 mL of warm blood

cardioplegia including 50 mg of lidocaine was administered to the donor heart. The cross clamp was then removed and the right atrial anastomosis was completed. The donor heart was reperfused for 30 min before attempting to separate from CPB. A dobutamine infusion (2.5 μg/kg/min) was commenced to provide inotropic support. After successful separation from CPB and assessment of hemodynamics and biventricular contractility the experiment was terminated.

Results

Brainstem death

Inflation of a subdural balloon-tipped catheter resulted in a rise in LV pressure and heart rate, and was accompanied by a rise in serum epinephrine and norepinephrine concentration After BSD there was a significant decrease in the LV ESPVR indicating a decline in load independent LV contractility (Table 1). Right ventricular contractility was preserved after BSD (Table 1). Thirty minutes after BSD their plasma concentration returned towards baseline levels (Figure 1A and Table 2). Load-dependent hemodynamic measurements demonstrated a significant increase in heart rate, cardiac output and rate of change in arterial pressure over time (dP/dt max) after BSD (Table 1).

Deceased donor heart resuscitation

After discontinuation of mechanical ventilation, hypoxic cardiac arrest and cessation of circulation occurred at 5.9 ± 2.8 min. Circulatory arrest was confirmed when the mean arterial pressure and the central venous pressure were equal, signaling the absence of blood flow. After observation of a 15-min warm ischemic period, extracorporeal blood perfusion was commenced on CPB (Figure 1B and Table 2). In the DCD donor plasma epinephrine and norepinephrine concentrations began to increase after discontinuation of mechanical ventilation and continued to increase throughout the period of circulatory arrest. At the end of the 15-min ischemic period the plasma norepinephrine concentration was 50-fold greater in the DCD donor than the peak concentration noted during BSD. Correspondingly the plasma epinephrine concentration was 30-fold greater than the peak value measured in the brain dead donor. Catecholamine levels continued to increase during the first 5 min of extracorporeal perfusion and then began to decline. After 30 min of reperfusion the norepinephrine and epinephrine concentrations in the DCD donor remained markedly elevated compared to baseline and were 2.5-fold and 2-fold greater, respectively than the peak levels seen after BSD (Figure 1). This catecholamine release was associated with a higher incidence of contraction bands in histological sections of the transplanted heart (Figure 2). Blood gas biochemistry after reperfusion of the DCD donor demonstrated marked metabolic abnormalities including metabolic acidosis and hyperkalemia (Table 3), this was corrected with appropriate pharmacological intervention as described earlier. Cardiac reanimation occurred within 5.1 ± 2.9 min of reperfusion. Spontaneous return of sinus rhythm occurred in three of seven donors, the remainder initially developed ventricular fibrillation and required internal DC defibrillation with a 20-J shock with restoration of sinus rhythm in all. CPB was continued for 41 ± 8 min before weaning and separation from extracorporeal circulation. Pressure–volume loops were acquired from both ventricles at baseline and following

Table 1: Invasive hemodynamic assessment of heart donors

Brainstem death	Pre-BSD	Post-BSD	p
Load independent contractility			
LV ESPVR	1.09 (0.54)	0.55 (0.19)	0.03
LV PRSW	47.5 (20.5)	36.4 (9.4)	0.42
RV ESPVR	0.34 (0.5)	0.37 (0.18)	0.85
RV PRSW	5.7 (1.9)	15.6 (8.6)	0.07
Load dependent measurements			
Heart rate (bpm)	75 (9)	92 (11)	0.02
LV dP/dt max (mmHg/sec)	815 (99)	1277 (332)	0.009
LV dP/dt min (mmHg/sec)	−997 (314)	−1132 (341)	0.49
Elastance (mmHg/mL)	1.7 (0.3)	1.4 (0.4)	0.26
Cardiac output (L/min)	2.9 (0.7)	4.5 (1.5)	0.04
LV end-systolic volume (mL)	58.7 (7.1)	70.5 (35.5)	0.49
LV end-diastolic volume (mL)	80.9 (19.4)	107.9 (39.9)	0.21
LV Stroke volume (mL)	39.3 (12)	49.4 (15)	0.23
Diastolic function			
LV EDPVR	0.11 (0.07)	0.16 (0.09)	0.39
RV EDPVR	0.04 (0.03)	0.03 (0.02)	0.63
DCD heart	**Prearrest**	**Postarrest**	**p**
Load independent measurements			
LV ESPVR	1.08 (0.61)	2.45 (1.18)	0.02
LV PRSW	28.1 (9.0)	45.3 (19.2)	0.04
RV ESPVR[1]	0.39 (0.12)	0.84 (0.27)	0.008
RV PRSW[1]	8.3 (3.9)	13.7 (6.3)	0.12
Load dependent measurements			
Heart rate (bpm)	80 (11)	123 (14)	<0.001
LV dP/dt max (mmHg/sec)	1127 (507)	1900 (849)	0.04
LV dP/dt min (mmHg/sec)	−1315 (434)	−1701 (709)	0.24
Elastance (mmHg/mL)	2.09 (0.69)	3.64 (1.17)	0.01
Cardiac output (L/min)	2.6 (0.7)	2.9 (1.0)	0.63
LV end-systolic volume (mL)	50.6 (11.1)	60 (7.7)	0.10
LV end-diastolic volume (mL)	76.2 (8.5)	74.9 (5.4)	0.75
LV Stroke volume (mL)	34.0 (9.5)	22.8 (6.2)	0.02
Diastolic function			
LV EDPVR	0.15 (0.10)	0.17 (0.08)	0.71
RV EDPVR[1]	0.07 (0.10)	0.07 (0.08)	0.92

Data are presented as mean (SD).
[1] n = 4.

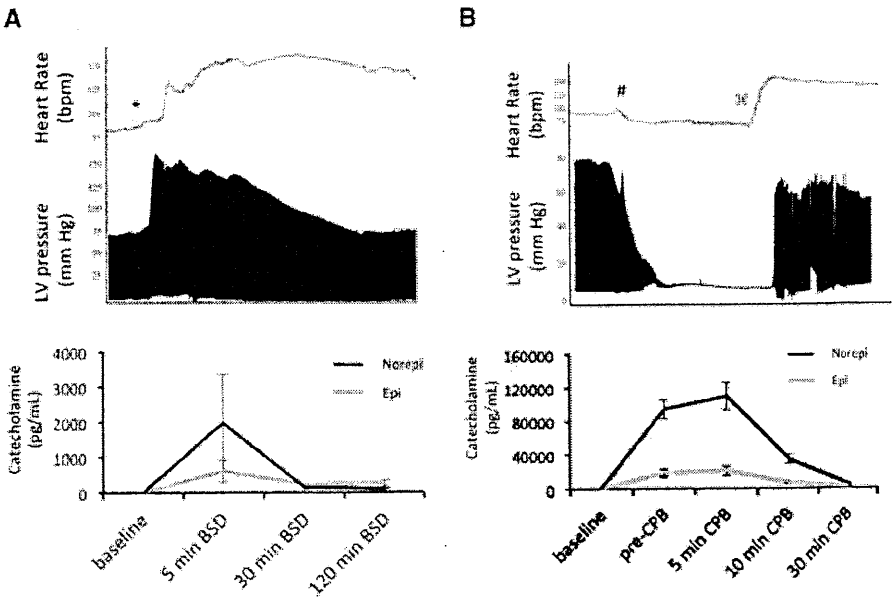

Figure 1: Hemodynamic parameters and serum catecholamine concentrations during intracranial balloon inflation leading to brainstem death (A) and donation after circulatory death (DCD) heart resuscitation (B) *Intracranial balloon inflation. #Ventilator off. †On cardiopulmonary bypass. Norepi, norepinephrine; Epi, epinephrine; BSD, brainstem death; CPB, cardiopulmonary bypass.

resuscitation (Figure 3). Detailed hemodynamic measurements are listed in Table 2. The resuscitated DCD heart exhibited significantly superior biventricular contractility compared to baseline (Table 1). Load-dependent measures of hemodynamic function revealed a significant increase in heart rate, dP/dt max and arterial elastance following resuscitation. The mean arterial blood pressure before circulatory arrest was not significantly different from that observed after weaning the resuscitated heart from extracorporeal perfusion (Table 2).

Table 2: Hemodynamic characteristics during BSD and DCD

	Pre-BSD	Brainstem death	Post-BSD
BSD (n = 8)			
MAP (mm Hg)	54.7 (4.8)	112.5 (25.1)	43.5 (4.1)
HR (bpm)	72 (8)	128 (57)	92 (25)
CVP (mm Hg)	3.3 (2.1)	6.8 (3.3)	3.9 (2.6)
LV dP/dt max (mm Hg/s)	1016 (461)	3532 (1156)	1549 (409)
Max LV systolic BP (mm Hg)	73.2 (2.2)	117.4 (21.5)	72.2 (6.4)
	Pre-arrest	**Circulatory arrest**	**Postarrest**
DCD (n = 8)			
MAP (mm Hg)	53.1 (7.3)	10.9 (4.2)	52.1 (16.5)
HR (bpm)	78 (9)	–	122 (15)
CVP (mm Hg)	3 (2)	9.8 (4.3)	4.9 (2.3)
LV dP/dt max (mm Hg/s)	1150 (313)	–	2028 (944)
Max LV systolic BP (mm Hg)	76.2 (6.2)	–	83.4 (11.1)

Data are presented as mean (SD).

Biventricular pressure–volume loop measurements

Direct comparison of load independent right and left ventricular contractility of the BSD heart and the DCD heart demonstrated significantly superior contractility of the latter. (Figure 3) There was an increase in the LV ESPVR and PRSW from baseline in the DCD heart and a decline in both of these indices after BSD. The RV ESPVR of the DCD heart was significantly greater than the BSD heart. Diastolic function was assessed using the linear EDPVR and did not reveal a significant difference in LV compliance between the two groups (Figure 3).

Cardiac cine-MRI

Biventricular function after DCD heart resuscitation was assessed using cardiac MRI (Figure 4). There was a trend

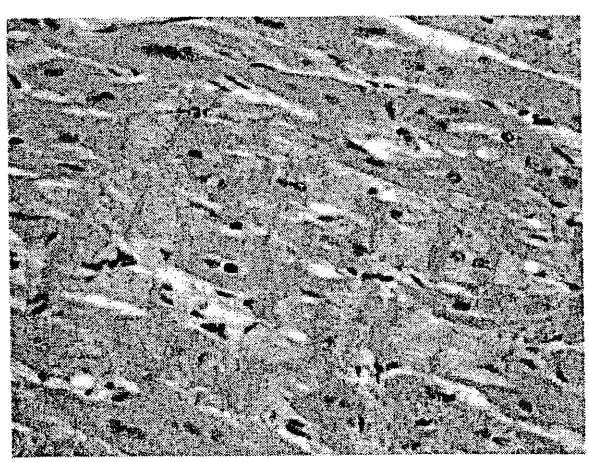

Figure 2: Histological section of myocardium from a resuscitated DCD heart, demonstrating contraction bands. Hematoxylin and eosin stain, 400× magnification.

Table 3: Biochemistry

BSD	Pre-BSD	Post-BSD
pH	7.48 (0.04)	7.39 (0.07)
$PaCO_2$ (mm Hg)	28.3 (2.5)	43.2 (10.4)
PaO_2 (mm Hg) (FiO_2 1.0)	339.4 (273.5)	465.6 (50.2)
SaO_2 (%)	99 (0.8)	99 (0.5)
Hct (%)	30 (2.8)	26.2 (3.7)
Hb (g/dl)	10.1 (0.9)	8.7 (1.2)
HCO^{3-} (mmol/L)	25.2 (2.1)	25.6 (2.6)
Base excess	1.7 (1.4)	0.6 (1.8)
Na^+ (mmol/L)	137 (3.1)	139 (3.7)
K^+ (mmol/L)	3.8 (0.5)	3.8 (0.5)
Cl^- (mmol/L)	105 (2.1)	107 (3.1)
Ca^{2+} (mmol/L)	1.3 (0.1)	1.18 (0.1)
Glucose (mmol/L)	8.6 (2.1)	12.2 (3.7)
Lactate (mmol/L)	0.9 (0.1)	2.5 (3.8)

DCD	Pre-arrest	Onset reperfusion	End reperfusion
pH	7.42 (0.03)	7.32 (0.11)	7.40 (1.0)
$PaCO_2$ (mm Hg)	39.1 (6.2)	43.7 (10.9)	39.1 (13.2)
PaO_2 (mm Hg) (FiO_2 1.0)	330.4 (165.5)	329.3 (108.9)	221.9 (87.8)
SaO_2 (%)	99 (1.2)	98 (0.9)	96 (6.1)
Hct (%)	25.6 (3.1)	26.2 (3.7)	32.1 (17.9)
Hb (g/dL)	8.8 (1.4)	7.4 (1.3)	8.9 (6.4)
HCO^{3-} (mmol/L)	25.6 (3.1)	22.4 (3.6)	23.1 (2.5)
Base excess	0.5 (2.7)	-4.2 (4.8)	-2.1 (2.1)
Na^+ (mmol/L)	141 (3.8)	143 (3.4)	141 (2.7)
K^+ (mmol/L)	3.9 (0.2)	6.1 (1.5)	4.2 (0.7)
Cl^- (mmol/L)	109 (3.1)	113 (1.9)	109.6 (2.4)
Ca^{2+} (mmol/L)	1.31 (0.1)	1.20 (0.1)	1.18 (0.06)
Glucose (mmol/L)	8.5 (8.3)	12.5 (7.0)	13.3 (7.5)
Lactate (mmol/L)	0.9 (0.4)	8.4 (1.8)	10.0 (1.9)

Data are presented as mean (SD).

towards a reduction in the LVEF following resuscitation but this did not reach statisical significance. LVEDV was not significantly altered compared to baseline. After resuscitaiton of the DCD heart there was a significant increase in RV volume suggesting RV dilatation and this was accompanied by a significant decrease in the RV EF (Figure 5B). During circulatory arrest the mean LVEDV in the arrested heart was 68 ± 7 mL with a mean RVEDV of 104 ± 17 mL. After BSD there was a decrease in both the left and right ventricular EDV in association with a significant increase in the LVEF (Figure 5A). Detailed measurements using cine-MRI of ventricular volumes and EF in both BSD and DCD hearts are listed in Table 4.

Magnetic resonance spectroscopy and measurement of high-energy phosphates

Myocardial energy metabolism was assessed using localized ^{31}P spectroscopy. Representative MRS spectra are depicted in Figure 6(A). After cardiac arrest in the DCD donor there was a significant increase in the ratio of myocardial inorganic phosphate to phosphocreatinine (P_i/pCr: pre 0.34 ± 0.13 vs. arrest 2.14 ± 0.91, $p = 0.002$) and a concomitant decrease in the ratio of phosphocreatinine to ATP (pCr/ATP: pre 3.53 ± 1.3 vs. post 2.04 ± 0.79, $p = 0.06$). After reperfusion and resuscitation there was normalization towards baseline of the P_i/pCr: 0.39 ± 0.25, $p = 0.69$ and an increase above baseline in the pCr/ATP: 5.8 ± 1.6, $p = 0.06$ (Figure 6B).

Posttransplant hemodynamic function

Orthotopic cardiac transplantation was undertaken in ten animals using hearts from both brainstem dead ($n = 5$) and DCD donors ($n = 5$). It was possible to wean all animals from CPB after transplantation confirming satisfactory early function of the graft. All animals in both groups required inotropic support with a low dose dobutamine infusion for maintenance of hemodynamics. There was no difference in load-independent biventricular contractility between transplanted BSD and DCD hearts (Figure 7). Mixed venous oxygen saturation after transplantation was normal in both groups indicating acceptable systemic oxygen delivery. (BSD 63.5 ± 2.3 vs. DCD 64.1 ± 3.1, $p = 0.61$). There was no difference in load-independent or dependent measures of hemodynamic function between the two groups (Table 5). MRI measurement of posttransplant biventricular function did not reveal any significant differences apart from a lower RV EF in the transplanted DCD heart.

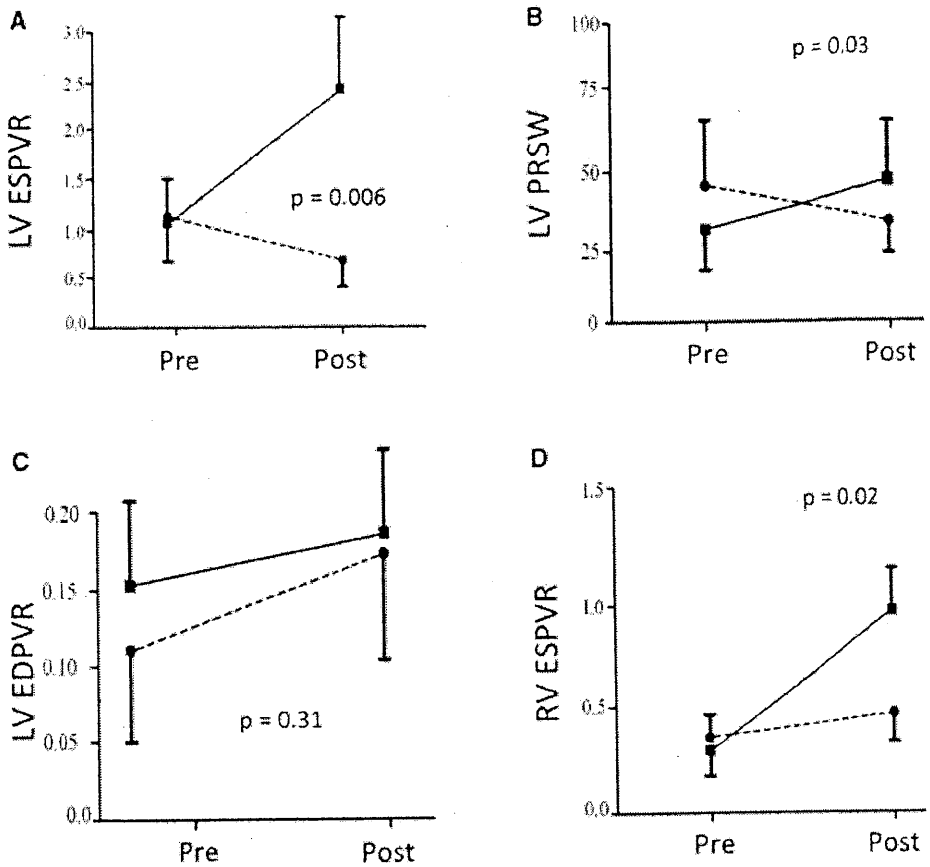

Figure 3: Assessment of left and right ventricular function of the BSD (•) and DCD (■) donor heart using conductance catheter. ESPVR, end systolic pressure volume relationship; EDPVR, end diastolic pressure volume relationship; PRSW, preload recruitable stroke work.

Discussion

The world's first heart transplant was performed using a heart resuscitated after cardiac arrest and declaration of death in the organ donor (4). Circulatory arrest was precipitated by discontinuation of mechanical ventilation. Cardiac resuscitation was achieved by rapid institution of CPB and perfusion of the coronary circulation with hypothermic oxygenated blood. The heart was successfully transplanted into a recipient and was able to support the circulation after separation from CPB. This method of donor heart procurement was subsequently abandoned after the widespread acceptance of BSD, which simplified the procedure for donor cardiectomy, avoiding the need to expose the heart to a period of warm ischemia. There has been renewed interest in the procurement of organs from DCD donors with the aim of expanding the pool of available organs for transplantation. Concerns have persisted over the feasibility of cardiac donation from this group of donors due to inevitable exposure of the organ to a period of ischemia. Using a porcine model of DCD donation we have demonstrated that the resuscitated DCD heart exhibits excellent biventricular contractility. Furthermore, we have demonstrated that high-energy phosphate concentration and metabolic function of these hearts is normal after resuscitation. Interestingly, we noted a dramatic increase in circulating catecholamines before and during reperfusion of the DCD donor at concentrations substantially greater than those observed during BSD.

Using MR spectroscopy we identified that the DCD heart recovers a near normal energy charge after resuscitation. Discontinuation of ventilation is followed by severe hypoxia and hypotension culminating in circulatory arrest. After cardiac arrest a period of warm ischemia must be endured by the organ before reperfusion can be established. Our findings confirm that depletion of high-energy phosphates occurs due to a shift to anaerobic metabolism during hypoxia and ischemia. Resuscitation and reperfusion of the DCD heart is followed by almost complete metabolic recovery of the organ with replenishment of energy stores. This confirms that the DCD heart can recover viability after cardiac arrest if reperfusion can be established.

The DCD heart exhibited excellent load-independent LV contractility after resuscitation. Both the LV ESPVR and PRSW increased significantly from baseline, suggesting superior contractility after resuscitation. Right ventricular contractility also increased significantly from baseline. In contrast, there was a reduction in the ESPVR one hour after

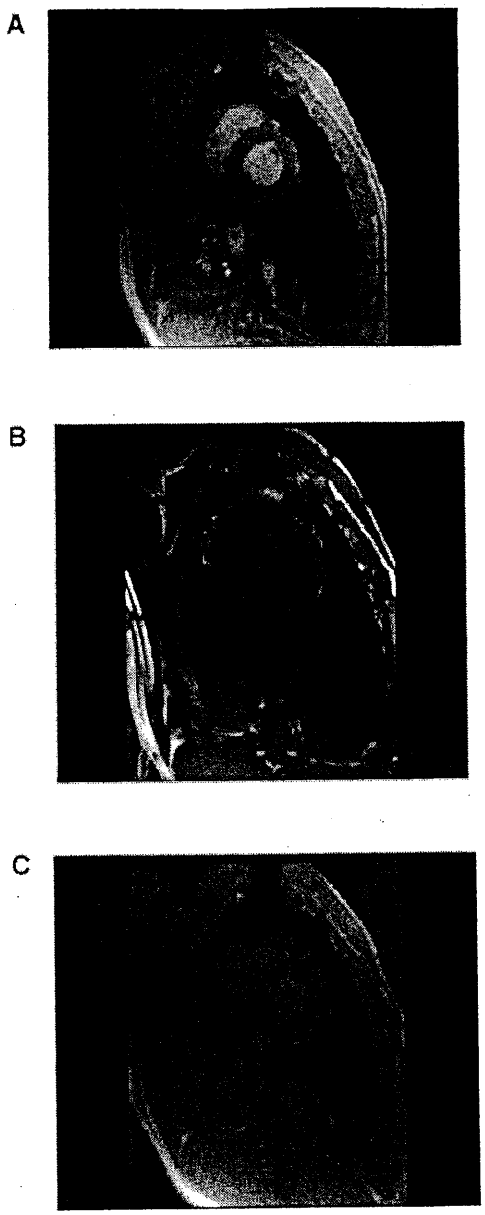

Figure 4: Representative MRI images of the DCD heart before (A), during (B) and after (C) circulatory arrest.

induction of BSD. A direct comparison of load-independent right and left ventricular contractility of the BSD and resuscitated DCD heart revealed significantly superior biventricular contractile function of the latter. In the brain dead donor a surge in catecholamine concentrations accompanies the rise in intracranial pressure that precedes ischemic compression of the brainstem. This sequence of events is associated with an initial hyperdynamic phase resulting in increased contractility, hypertension and tachycardia (15,16). However, this phase subsides soon after brain death and is followed by a decline in catecholamine levels and progressive hypotension. In our study, serial measurement of plasma epinephrine and norepinephrine concentrations in the BSD animals were in accordance with previous observations. Exposure of the donor heart to excessive catecholamine levels is associated with hemodynamic dysfunction and both direct and indirect myocardial injury (17–19). We report the novel discovery of a substantially more profound rise in catecholamines within the reperfused DCD donor. Serum epinephrine and norepinephrine concentrations began to increase after termination of ventilation and continued to increase throughout circulatory arrest and the initial period of reperfusion. There was an approximate 50-fold increase in norepinephrine and 30-fold increase in epinephrine concentrations compared to peak levels of these catecholamines observed in the BSD donor. With continued reperfusion the levels began to decline, however even after 30 min of reperfusion they remained markedly elevated compared to baseline. Contractile function of the DCD heart is likely to have been augmented by the enhanced internal inotropic state of the DCD donor. The implications of exposure of the myocardium to such dramatically elevated catecholamine levels requires further investigation. Despite concerns over catecholamine mediated myocardial injury, resuscitated DCD hearts were successfully transplanted into five recipient animals and were capable of supporting the recipient circulation.

Using MRI we identified a significant decrease in RV EF after resuscitation of the DCD heart in association with RV dilatation. However, load independent RV contractility assessed via the ESPVR was seen to be significantly greater than baseline. This discrepancy highlights the importance of assessing contractile performance independent of loading conditions. The mode of death of the DCD donor is hypoxic cardiac arrest. Before cessation of circulation there is an agonal period during which organs are exposed to hypoxic perfusion. During this period ventricular dilatation occurs as a consequence of circulatory loading as the arterial and venous pressures equalize. The production of such a circulatory load and its potential for causing myocardial damage is recognized. Osaki and colleagues compared function of DCD hearts transplanted after the induction of cardiac arrest by either asphyxiation or exsanguination (9). They reported that circulatory load associated with asphyxiation led to impaired LV function of transplanted DCD hearts. They noted an increase in the LVEDV to 132% of baseline after circulatory arrest in the asphyxiated donor. In contrast to our study, they did not reperfuse and resuscitate the DCD heart within the donor. After exposure of the organ to a 30-min warm ischemic period the heart was excised. This was followed by continuous aortic root and coronary perfusion of blood cardioplegia using an *ex vivo* perfusion apparatus. After an hour of reperfusion orthotopic transplantation of the heart into a recipient animal was undertaken. All animals were successfully weaned from CPB using an epinephrine infusion. They reported

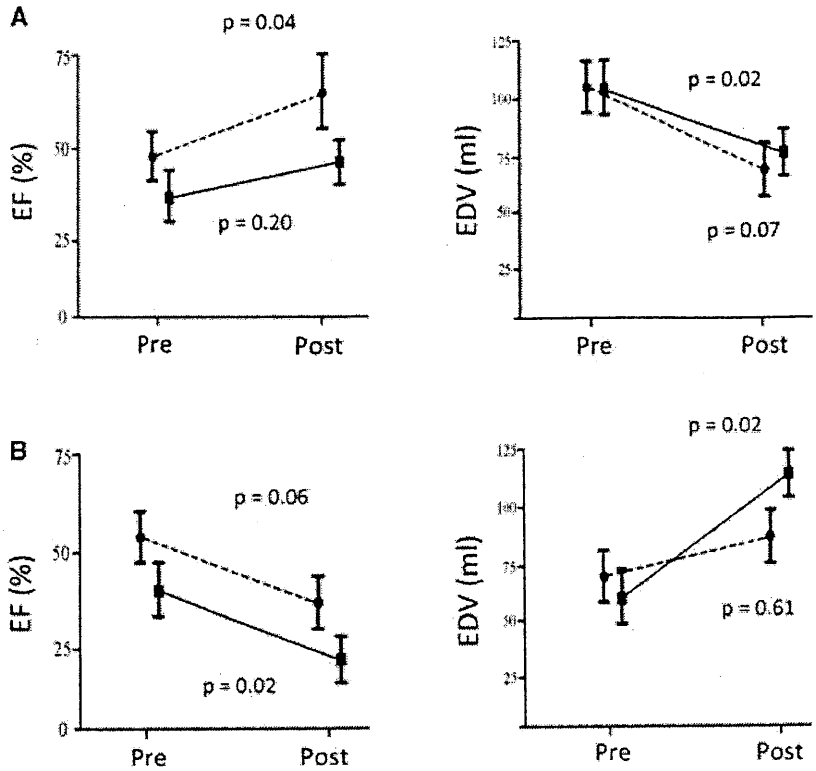

Figure 5: MRI assessment of LV (•) and RV (■) ejection fraction (EF) and end-diastolic volume (EDV) in BSD (A) and DCD (B) donor heart.

that LV E_{max}, LV max dP/dt and LV min dP/dt were significantly worse in transplanted asphyxiatied donor hearts than exsanguinated donor hearts (9). We were not able to identify a difference in LV max dP/dt or LV min dP/dt in hearts transplanted from either DCD or brainstem dead donors. Furthermore we did not observe a significant increase in LVEDV or impairment of LV function after resuscitation of the DCD heart. Our predominant finding was that of RV dilatation and apparent impairment of RV function suggested by a marked reduction in RV EF. In contradiction to these findings was the observation of supranormal load-independent RV contractility of the DCD heart after resuscitation according to the RV ESPVR. It is likely that due to the capacitance of the venous circulation and increase in central venous pressure during circulatory arrest, the RV is more likely to be injured as a result of circulatory loading. Previous work has demonstrated that the RV may incur greater injury after exposure to catecholamines than the LV in the brainstem dead donor (20).

Table 4: Cardiac volumes and function obtained from cine-MRI after brainstem death and DCD heart resuscitation

Brainstem death (n = 8)	Pre-BSD	Post-BSD	p
LVEDV (mL)	101 (16)	70 (18)	*0.02*
LVESV (mL)	52 (9)	23 (8)	*<0.001*
LVEF (%)	48 (10)	67 (14)	*0.04*
RVEDV (mL)	101 (20)	75 (22)	0.07
RVESV (mL)	66 (16)	42 (22)	0.07
RVEF (%)	35 (8)	45 (13)	0.20
CO (L/min)	4.3 (1.1)	4.1 (1.7)	0.86
DCD donor (n = 8)	**Pre-arrest**	**After reperfusion**	**p**
LVEDV (mL)	73 (14)	78 (22)	0.61
LVESV (mL)	36 (9)	52 (15)	0.05
LVEF (%)	50 (16)	34 (13)	0.06
RVEDV (mL)	68 (17)	107 (25)	*0.02*
RVESV (mL)	43 (9)	85 (26)	*0.001*
RVEF (%)	36 (6)	22 (10)	*0.03*
CO (L/min)	3.4 (0.8)	2.9 (1.2)	0.38

Data are presented as mean (SD).

A continuing decline in the number of organs available for heart transplantation has led us and others to re-evaluate the utility of DCD hearts for transplantation. Successful pediatric transplantation from DCD donors has recently been reported with survival at 6 months postoperatively (21). We have demonstrated in an adult DCD donor that extracorporeal perfusion of the arrested heart is followed by sufficient recovery of contractile function to support the circulation (14). Our current investigation has confirmed that hearts resuscitated after circulatory arrest are viable with evidence of normal energy metabolism. Load-independent measures of contractility confirm excellent biventricular function of the DCD heart. However, we have also observed some unexpected and potentially important

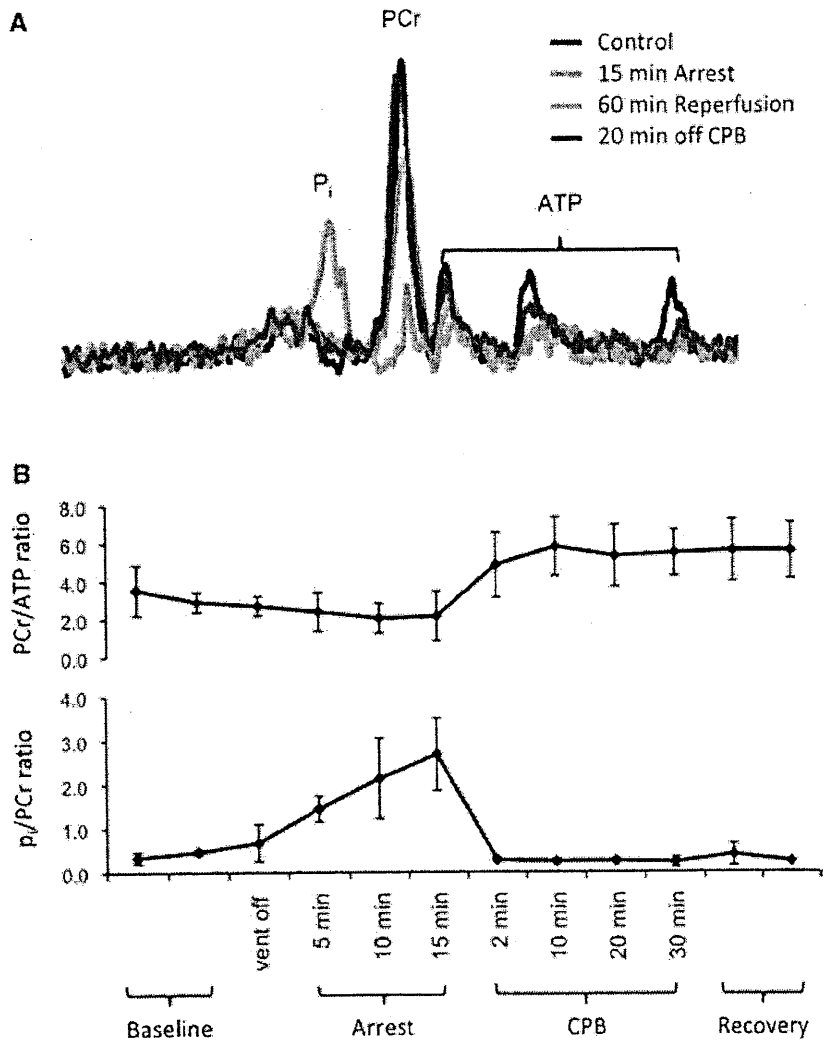

Figure 6: High energy phosphate metabolism of the DCD heart after circulatory arrest and resuscitation. (A) Representative ^{31}P magnetic resonance spectra during circulatory arrest and reperfusion of the DCD heart *in vivo*. (B) Average PCr/ATP and p_i/PCr ratios during circulatory arrest and reperfusion of the DCD heart *in vivo*. PCr, phosphocreatine; p_i, inorganic phosphate; CPB, cardiopulmonary bypass.

findings that require further evaluation before clinical use of DCD hearts can be advocated without reservation. Circulatory arrest and reperfusion of the DCD donor is associated with a torrential rise in plasma catecholamines, dwarfing the levels seen after BSD. Catecholamine mediated injury of the brainstem dead donor heart has been the subject of considerable investigation. This is often cited as being responsible for poor donor heart function reported at the time of evaluating the organ for transplantation. Furthermore, in the BSD donor this catecholamine surge is associated with a marked inflammatory response that is reported to have immunomodulatory effects that can predispose to acute and chronic rejection. Consequently, the impact of exposure of the DCD heart to markedly elevated epinephrine and norepinephrine levels requires detailed assessment. Secondly, there is suggestion of right ventricular impairment of the DCD heart. This in itself may partly be due to catecholamine-mediated injury, similar to that observed in the BSD donor RV (20). RV dilatation due to circulatory loading after hypoxic cardiac arrest is another possible mechanism of injury. In the context of clinical heart transplantation, where perioperative RV dysfunction is a well-recognized issue, particularly in the setting of elevated recipient PVR, the finding of a tenuous RV in the DCD heart warrants a thoughtful approach to the clinical application of DCD heart transplantation. Nevertheless, despite some caution raised by these findings, hearts from DCD donors were successfully weaned from CPB after orthotopic transplantation into recipient animals who had normal PVR. Furthermore, posttransplant biventricular contractility and hemodynamic function was not significantly different to hearts transplanted from conventional BSD donors. To optimize function of the transplanted heart, efforts must be directed towards designing protocols for resuscitation, preservation and implantation that are specifically tailored to the DCD heart. In this respect our data

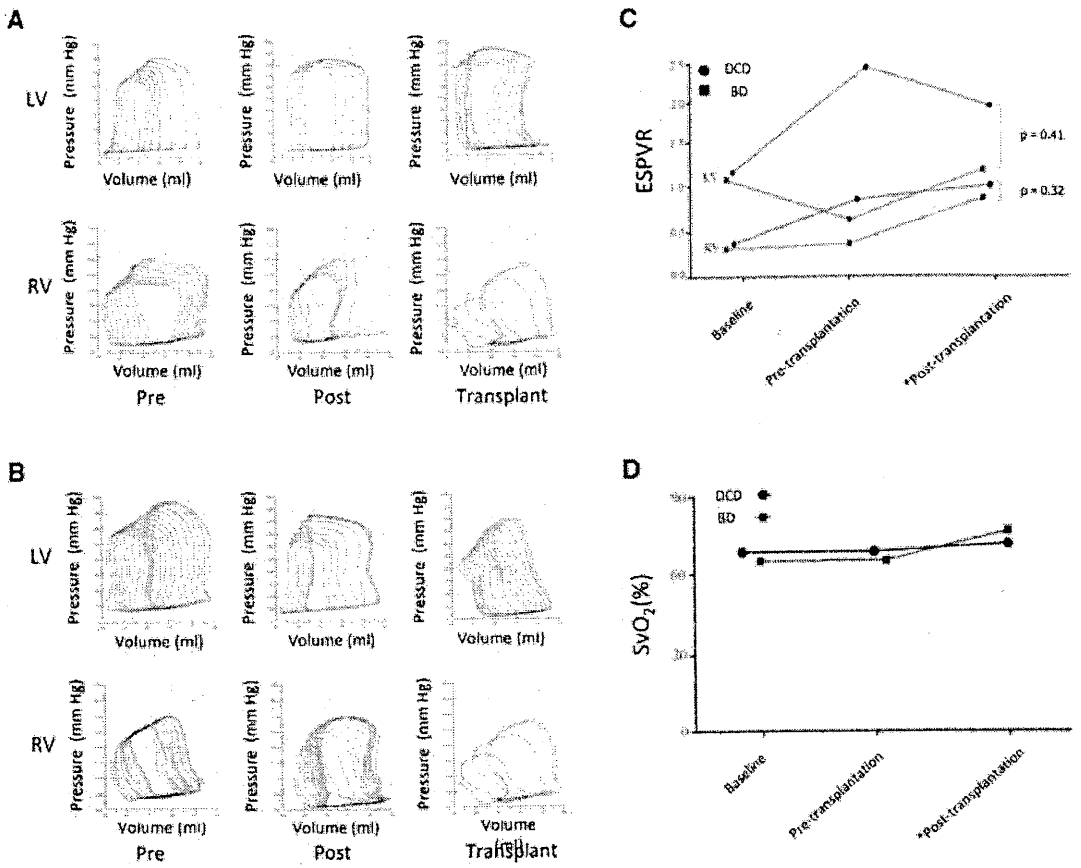

Figure 7: (A) Representative pressure–volume loops across BSD and heart transplantation. (B) Representative pressure–volume loops across DCD and heart transplantation. (C) Average end systolic pressure volume relationship (ESPVR) across BSD or DCD and heart transplantation. (D) Change in mixed venous oxygen saturation (SvO_2) across BSD or DCD and heart transplantation. *2.5 mcg/kg/min dobutamine.

suggests that an alternate strategy, such as *ex vivo* machine perfusion of the DCD donor heart, may be advantageous to *in vivo* reperfusion as it may help to avoid exposure of the organ to profoundly elevated catecholamine levels. *Ex vivo* perfusion has been studied to a limited degree, with respect to prevention of ischemia-reperfusion injury and optimizing recovery of cardiac function after resuscitation (22). Continuous machine perfusion would also avoid the need for cold storage. The benefits of avoiding cold storage have been reported in an experimental model of DCD liver transplantation. Reddy and colleagues identified that warm ischemia and subsequent cold ischemia during storage can result in additive injury and severe organ damage (23). It may also be beneficial to incorporate methods that allow for continuous reperfusion of the organ even during implantation as undertaken by Osaka and colleagues in their experimental model (9,11).

There are important limitations of our study. First, we heparinized the animals and cannulated the heart for CPB, maneuvers that are not possible in the clinical context. Secondly, the period from cessation of mechanical ventilation to cardiocirculatory arrest was relatively short, being only 5.9 ± 2.8 min. This was followed by a 15-min stand-off period. Therefore, the total time was shorter than is typically seen in the clinical context. Further experiments are required to determine the maximum period of warm ischemia that is possible.

In conclusion, continued investigation is paramount for the translation of experimental evidence, supporting the feasibility of DCD heart transplantation, into a clinical program. If the DCD donor heart can be confirmed as suitable for use in transplantation it has the potential to produce an immediate and marked expansion of the donor pool. In 1967 heart transplantation was pioneered using

Table 5: Posttransplant hemodynamic function

Orthotopic heart transplantation	DCD (n = 5)	BSD (n = 5)	p
Load-independent measurements			
LV ESPVR	1.77 (0.96)	1.04 (0.13)	0.43
LV PRSW	80 (26)	54 (19)	0.21
RV ESPVR[1]	0.90 (0.28)	0.42 (0.18)	0.32
Load-dependent measurements			
MAP (mm Hg)	48.0 (8.0)	54.2 (8.6)	0.33
HR (bpm)	115 (24)	101 (10)	0.31
Max LV systolic BP (mm Hg)	84 (16)	77 (15)	0.63
LV dP/dt max (mm Hg/s)	1585 (172)	1535 (421)	0.83
LV dP/dt min (mm Hg/s)	-1234 (231)	-1557 (477)	0.27
Diastolic function			
LV EDPVR	0.12 (0.04)	0.07 (0.02)	0.15
RV EDPVR[1]	0.04 (0.03)	0.05 (0.03)	0.25
MRI measurements			
LVEDV (mL)	44 (13)	44 (13)	0.99
LVESV (mL)	18 (7)	23 (16)	0.73
LVEF (%)	58 (9)	53 (26)	0.70
RVEDV (mL)[1]	53 (19)	64 (12)	0.27
RVESV (mL)[1]	43 (9)	39 (15)	0.30
RVEF (%)[1]	10 (11)	25 (10)	0.04
CO (L/min)	3 (0.8)	2.4 (0.6)	0.32

Data are presented as mean (SD).
[1]n = 3.

a DCD donor, 40 years later we believe this strategy may be instrumental in salvaging this therapy from the serious obstacle of donor organ shortage.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by the *American Journal of Transplantation*.

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