Characterizing Cardiac Donation After Circulatory Death: Implications for Perfusion Preservation

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Background. Donation after circulatory determination of death (DCDD) involves variable definitions of death among hospitals, and DCDD hearts are not generally considered for transplantation. The definition can affect ischemic times, and machine perfusion preservation appears promising for recovery of DCDD hearts. The purpose of the current study was to investigate the agonal phase of DCDD donors and evaluate retrograde perfusion preservation of DCDD donor hearts in a large animal model of cardiac transplantation.

Methods. Ten canines were anesthetized and then disconnected from mechanical ventilation. Time to loss of pulse (systolic blood pressure < 50 mm Hg), loss of pressure, and asystole or fibrillation were recorded. Five minutes after asystole, hearts were exposed and arrested with 1 L of University of Wisconsin Machine Perfusion Solution. Eight hearts were cold preserved for 4 hours by retrograde machine perfusion or static storage (n = 4/group), then reimplanted and reperfused for 6 hours.

At any given time, there are nearly 4,000 patients on the heart transplant waiting list in the United States, with only 2,000 to 2,500 actually receiving organs annually [1]. Despite the large need, nearly 60% of potential standard criteria donors and more than 70% of all cardiac donors are rejected for heart transplantation [2]. Donation after cardiac death (DCD) has increased the donor pool for abdominal organ transplantation with acceptable results [3, 4]. Although the first heart transplant done by Christiaan Barnard in 1967 used a DCD donor [5], with the traditional definition of irreversible loss of cardiac function, this has generally not been considered for cardiac donation [6]. However, several transplants have been done in pediatric patients using a very short waiting period (2 minutes or less) after declaration of death, creating significant controversy surrounding the ethics and protocol of DCD [7]. More recently, there has been a shift in terminology from DCD to donation after circulatory determination of death (DCDD) to reflect that cessation of effective circulation is the terminal event in these donors [8]. The physiologic parameters that constitute circulatory death remain contentious, with recommendations to adjust and standardize the definition of circulatory death and the waiting period before organ harvest [8]. The definition of circulatory or cardiac death remains variable, ranging from loss of pulse (systolic blood pressure ≤ 50 mm Hg) to loss of pressure waveform (as measured by an arterial line) to asystole by electrocardiographic monitor [8–11]. Additionally, the time to start the procurement after declaration of death is also debated. Some ethicists suggest a waiting period of 10 minutes, whereas recommendations from the Institute of Medicine (5 minutes), and transplant societies (2 minutes) are shorter because spontaneous return of circulation has not been documented past 60 to 75 seconds [8, 10]. These issues are of particular importance when considering DCDD for cardiac transplantation because of the reduced ischemic tolerance of donor hearts. Any advancement in the preservation techniques that improve or even resuscitate donor hearts may allow for recovery of DCDD organs, even if the most stringent definitions of circulatory death are applied.

Machine perfusion preservation is one strategy that shows promise for recovery of cardiac donors after...
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fluence of improved outcomes after kidney transplantation from extended and DCDD donors are well documented [12, 13]. This technique is gaining increased interest for preservation of cardiac donors. A warm, beating heart technique using the Organ Care System (TransMedics, Inc, Andover, MA) has gained CE mark approval and is currently undergoing clinical trials in the United States [14]. We have previously investigated hypothermic machine perfusion preservation of cardiac donors in a variety of models, including transplantation after both standard and long-term storage intervals. In these experiments we demonstrated improved cardiac function, lower lactate to alanine ratios, increased high-energy phosphate stores, and reduced apoptosis [15–18]. Most of these studies used antegrade perfusion in which the perfusate was delivered into the aorta and then to the coronary arteries. We noted that under some conditions, aortic insufficiency and nonnutrient flow could occur, reducing the effectiveness of this technique [19]. We since have explored the use of retrograde perfusion through the coronary sinus to avoid the potential for aortic valve incompetence. Retrograde cardioplegia is used routinely for myocardial preservation during cardiac surgery [20–22]. Initial results in a 12-hour storage model of cardiac transplantation have been favorable with this technique [23], despite concerns about right ventricular protection with retrograde cardioplegia [24, 25].

The purpose of this study was to investigate the agonal phase of DCDD and compare the functional recovery of implanted hearts preserved by either conventional static storage or retrograde machine perfusion, using a large animal model of cardiac transplantation. We hypothesized that retrograde machine perfusion would result in better recovery of DCDD hearts than static storage, even under the most stringent of cardiac or circulatory death definitions.

Material and Methods
Experimental Protocol
The protocol used in this study was approved by the Institutional Animal Care and Use Committee at the University of Texas Southwestern Medical Center. All animals were treated in accordance with guidelines set forth in the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 86-23, revised 1996).

Ten adult mongrel dogs were used to examine the agonal phase of the DCDD model. Eight of these hearts were then paired with 8 recipient animals divided into two groups, hypothermic static storage (static, n = 4) and retrograde machine perfusion preservation (RP, n = 4). University of Wisconsin Machine Perfusion Solution (Trans-Med Corp, Elk River, MN) was used for preservation in both groups. In the RP group, perfusate was delivered through a catheter secured in the coronary sinus using a machine perfusion system (LifeCradle; Organ Transport Systems, Inc, Frisco, TX). Hearts were stored for 4 hours, then implanted into the recipients and reperfused for 6 hours before explantation for tissue samples.

Anesthetic Protocol
Anesthesia was induced with 4.4 mg/kg of tiletamine (Telazol; intramuscular) along with 0.07 mg/kg of atropine intramuscularly. Animals were intubated and ventilated with 100% oxygen at a rate of 10 breaths/min, tidal volume of 10 mL/kg, and positive end-expiratory pressure of 5 cm H2O. Ventilator adjustments were made to maintain a pH of 7.35 to 7.45, PCO2 of 35 to 45 mm Hg, and oxygen saturation greater than 95%. Anesthesia was continued with isoflurane at 1% to 4%. Electrocardiogram, central venous pressure, and arterial pressure were monitored.

Agonal Phase and Donor Protocol
After induction of anesthesia, a sternotomy was performed to expose the heart, which was then instrumented with a left ventricular (LV) pressure catheter and sommicroscopy crystals were placed in the long and short axis of the LV. Baseline functional data were obtained, and the animals were then medicated with 1 mg/kg of morphine to emulate the comfort measures offered to human DCDD donors, as well as 300 U/kg of heparin. The ventilator was then disconnected with the animal adequately anesthetized to remain apneic. Rate-pressure product and preload recruitable stroke work (PRSW) were obtained at 1 minute, followed by 2-minute intervals after cessation of ventilation. Time to loss of pulse (systolic blood pressure <50 mm Hg), loss of pressure waveform, and loss of electrical activity or fibrillation were measured. After electrocardiographic silence or ventricular fibrillation, we waited 5 minutes to replicate a commonly used DCDD wait period, as well as an additional 7 minutes to account for the time it would normally take to enter the chest and expose the heart after death. We then harvested 8 of the 10 donor hearts and randomly assigned them to RP or static groups. The perfusion device was attached to a silicone elastomer catheter (Medtronic Inc, St. Paul, MN) secured in the coronary sinus with a pursestring suture. The balloon was not inflated to avoid obstruction of cardiac veins draining the right ventricle. Oxygenated University of Wisconsin Machine Perfusion Solution was perfused continuously at a rate of 20 mL/100 g myocardium per minute and a temperature 5° ± 2° C, based on prior canine retrograde perfusion data [24]. A small catheter was secured in the aortic root to obtain serial coronary effluent samples for measuring oxygen level, lactate concentration, and pH. Heart weights were obtained at baseline, after storage, and after reperfusion.

Recipient Protocol
Anesthesia was induced as previously described. After heparin administration (300 U/kg), the animal was cannulated and placed on cardiopulmonary bypass (CPB). The heart was excised in coordination with the end of the storage period, and a bicaval orthotopic transplant.
technique was used for donor implantation. Methylprednisolone (1 g) was given before removal of the aortic cross-clamp. Defibrillation with 5 to 20 J was used as needed, and inotropic support was started with 0.05 μg · kg⁻¹ · min⁻¹ epinephrine, 2 U/h vasopressin, and 5 μg · kg⁻¹ · min⁻¹ dobutamine before separating from CPB. The LV pressure catheter was replaced, and the sonomicrometry crystals were reconnected. After an hour of reperfusion, attempts were made to wean the recipient animal from CPB. Hourly pressure-volume (PV) loops for PRSW were obtained for the 6-hour reperfusion period. Blood for cardiac enzyme release and ventricular tissue samples for terminal deoxynucleotidyl transferase-mediated UTP nick end label (TUNEL) assay and myocardial water content were taken at the completion of each experiment.

Measurement of Ventricular Performance
The PRSW, calculated from PV loops, was used as a load-independent quantifier of LV function [26, 27]. A micromanometer-tipped catheter (Millar Instruments, Houston, TX) was passed through the apex into the LV to measure pressure, and volume (dimension) of the LV was obtained using four sonomicrometry crystals (Sonometrics Corp, London, Ontario, Canada) attached to the subendocardium in the minor and major axes. The catheter was removed for storage, and the crystals were left in situ. Data for the PV loops were collected at a rate of 250 Hz, digitized, and stored on a computer for later analysis using commercially available software (Sonolab and CardioSOFT; Sonometrics). To obtain emptying curves (PV loops over a range of filling conditions), either blood was drained into the CPB reservoir or the vena cavae were manually occluded. The PRSW was calculated by plotting the stroke work (PV loop integral) against the end-diastolic volume and taking the slope of the regression.

Measurement of Myocardial Water Content
Ventricular tissue taken at the end of the experiment was weighed and placed in a drying oven, with daily weights taken until a constant weight was achieved (dry weight). The formula (wet weight – dry weight)/wet weight was used to calculate myocardial water content.

Measurement of Cardiac Enzymes
Creatine kinase and troponin levels (Cobas Analyzer, Roche Diagnostics, Indianapolis, Indiana) were measured from blood samples taken just before explantation. The creatine kinase level was measured by enzymatic activity level using absorption photometry, with results given as international units per liter. Troponin T was evaluated using electrochemiluminescence immunoassay and reported as nanograms per milliliter.

Myocardial Cell Death
Separate, duplicate right ventricular and LV tissue samples were harvested after explantation for assessment of cell death by TUNEL. Tissue was fixed with 4% paraformaldehyde. Subsequent paraffin processing, embedding, and sectioning were performed by standard procedures [28].

Terminal deoxynucleotidyl transferase-mediated UTP nick end labeling was performed according to the Promega DeadEnd Fluorometric TUNEL System protocol [29]. Dead cells were labeled with fluorescein, and the sections were counterstained with propidium iodide. Ten 200× fields per slide were examined to quantify total TUNEL-positive nuclei. Results were indexed to the total number of nuclei from the same field. Images were obtained in a blinded fashion and subsequently analyzed with ImageJ image processing software (National Institutes of Health, Bethesda, MD).

Statistical Analysis
Results are reported as mean ± standard error of the mean (SEM), and calculations were performed using commercially available statistical software (SigmaStat, Chicago, IL). The groups were compared using a two-sided Student’s t test or analysis of variance when appropriate. A repeated-measures analysis of variance was applied for variables measured at multiple time points during the course of the experiment. A probability value of less than 0.05 was considered significant.

Results
The donor hearts were able to maintain a stable rate-pressure product up to 7 minutes after cessation of ventilation, at which point the values significantly and steadily declined (p < 0.05; Fig 1). The PRSW followed a similar pattern, with hearts maintaining a stable level until 7 minutes, and then decreasing by an average of 35% from baseline (p < 0.05 compared with baseline). After 7 minutes the LV function was insufficient to obtain PV loops and calculate a PRSW. The time to loss of pulse and loss of pressure waveform were both relatively early and consistent, averaging around 8 and 10 minutes, respectively; however, the time to electrical silence or fibrillation was significantly higher than the other measures and demonstrated greater variability (p < 0.05; Table 1).

The agonal phase and time to cross-clamp placement were not different between groups. Storage times, implant times, and total ischemic times were also similar (Table 2). During the storage interval, oxygen consumption and lactate levels were elevated for the first 30 minutes in RP hearts. Both parameters decreased and leveled off for the rest of the 4-hour perfusion period (Fig 2). After implantation and reperfusion, the myocardial function as measured by PRSW was higher in the RP hearts than in static hearts (p < 0.05 for hours 1, 2, 3, and 5). Additionally, the PRSW in RP hearts remained similar to baseline levels, whereas function in the static hearts decreased below their baseline (p < 0.05 for hours 1, 3, and 6; Fig 3).

All RP hearts were able to separate from CPB by the end of the first hour and remained off CPB for the entire 6-hour reperfusion interval. Three of the four static hearts initially were able to wean from bypass, but two of four
hearts required a return to CPB by the end of the experiment.

Cardiac enzyme leak was higher in the static group at the end of the experiment; however, this difference was only significant for total creatine kinase ($p < 0.05$ for creatine kinase; $p = 0.16$ for troponin T; Fig 4). There was weight gain in both groups after storage; however, the RP hearts then lost the weight during reperfusion, whereas the static hearts gained additional weight. The water content of both ventricles measured at the end of the experiment was not different between the groups (Table 3).

Staining by TUNEL was used to quantify cell death on tissue taken at the end of the reperfusion interval. We did not attempt to differentiate between apoptosis and necrosis on the basis of nuclear morphology, but instead focused on the total number of dead cells. When comparing the fraction of positive cells in both ventricles, static hearts had a higher fraction of TUNEL-positive cells than did RP hearts ($p < 0.05$; Fig 5).

**Comment**

Donation after circulatory determination of death has rarely been used for cardiac transplantation even though the donor for the first successful heart transplant by Christiaan Barnard was procured after cardiocirculatory death was determined in an operating room adjacent to the recipient [5]. Other reports also illustrate that under stringent, controlled conditions and brief periods of ischemia, DCDD for cardiac transplantation is possible [7]. Widespread application up until this point is unlikely for a variety of reasons including unpredictable time to DCDD, donor hospital policies, logistics of the procurement, and prolonged ischemic times. For example, unlike a standard brain-dead donor when the implanting team would proceed with anesthetic induction and dissection after visualization of the heart, thus minimizing storage time, in a DCDD procurement, the recipient team cannot move forward until it is notified that the donor has died within an acceptable time.

The cardiac donor pool has not changed significantly in more than a decade, and a lack of suitable donors remains a major limitation for cardiac transplantation. Although waiting list mortality has decreased recently mainly because of the development of durable LV assist devices, the number of patients on the heart transplant waiting list remains nearly double the number of transplants performed annually in the United States [1]. Even among standard criteria donors, the conversion rate is still only a little more than 40%. Donation after circulatory determination of death has gained increasing acceptance in recovery of abdominal organs and, to some extent, lung transplantation but has not been seriously considered for increasing the cardiac donor pool. Our experiments suggest that machine perfusion preservation is a strategy that may allow for reliable recovery of DCDD for heart transplantation. The average time to cross-clamp in this study would capture more than 90% of all liver donors

**Table 1. Agonal Phase Timeline**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SEM</th>
<th>Range</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loss of pulse (min)</td>
<td>7.9 ± 0.5</td>
<td>5–11</td>
<td>0.002, 0.0007 a</td>
</tr>
<tr>
<td>Loss of pressure (min)</td>
<td>10.2 ± 0.4</td>
<td>9–13</td>
<td>0.0022</td>
</tr>
<tr>
<td>Electrical silence/fibrillation (min)</td>
<td>26.9 ± 3.8</td>
<td>11–43</td>
<td></td>
</tr>
</tbody>
</table>

a Versus loss of pressure and electrical silence/fibrillation, respectively.

**Table 2. Ischemia Timeline**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Static</th>
<th>Perfused</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agonal phase time (min)</td>
<td>26 ± 6</td>
<td>23 ± 3</td>
<td>0.70</td>
</tr>
<tr>
<td>Time to cross-clamp (min)</td>
<td>34 ± 7</td>
<td>34 ± 6</td>
<td>0.99</td>
</tr>
<tr>
<td>Cold storage time (min)</td>
<td>255 ± 10</td>
<td>264 ± 9</td>
<td>0.49</td>
</tr>
<tr>
<td>Implantation time (min)</td>
<td>51 ± 3</td>
<td>45 ± 2</td>
<td>0.17</td>
</tr>
<tr>
<td>Total ischemic time (min)</td>
<td>340 ± 14</td>
<td>343 ± 13</td>
<td>0.85</td>
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SEM = standard error of the mean.
based on a decade-long review of the Organ Procurement and Transplantation Network DCD liver transplantation experience from 2010 [3]. Considering that fewer than 20% of potential DCDD donors are even evaluated, the potential to increase the cardiac donor pool is substantial even if only younger donors are accepted [4].

The current study was designed to characterize the agonal phase of the DCDD cardiac donor and evaluate the ability to successfully transplant donor animals after a storage interval that would permit recovery of donors over currently acceptable procurement distances. Our data suggest that in an apneic or near-apneic donor, loss of pulse and arterial waveform are fairly consistent events but time to electrical silence varies widely. Systolic function as measured by PRSW and rate-pressure product was preserved until loss of pulse, although some diastolic dysfunction was noted as evidenced by increasing diastolic pressures, right ventricular dilatation, and increasing end-diastolic volume (data not shown). We chose the most stringent definition of DCDD because despite recommendations by the various societies, many hospitals either have no policy to define circulatory death or use asystole as the determining event [11]. We demonstrated that the definition of cardiac death may have important implications for recovery of these donors, but independent of the definition of death, donor hearts could be successfully transplanted after retrograde machine perfusion preservation whereas recovery after conventional hypothermic static storage was unpredictable.

Development of myocardial edema is a potential concern for machine-perfused hearts. In the current study, perfused hearts experienced approximately a 10% weight gain during the storage interval. This increase dissipated by the end of the reperfusion period, and myocardial water content at that point was not different between static storage and perfused hearts. This experience is consistent with our previous studies using different preservation conditions, which suggested that despite significant weight gain during the storage interval (in some cases up to 30%), reperfusion function was not adequate for transplantation.

Table 3. Weight Changes and Water Content

<table>
<thead>
<tr>
<th>Variable</th>
<th>Static</th>
<th>Perfused</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poststorage weight – initial (g)</td>
<td>13 ± 10</td>
<td>22 ± 11</td>
<td>0.59</td>
</tr>
<tr>
<td>Postreperfusion weight – initial (g)</td>
<td>18 ± 13</td>
<td>-3 ± 6</td>
<td>0.2</td>
</tr>
<tr>
<td>RV water content (%)</td>
<td>81.7 ± 0.4</td>
<td>80 ± 0.7</td>
<td>0.16</td>
</tr>
<tr>
<td>LV water content (%)</td>
<td>78.8 ± 1.1</td>
<td>79.7 ± 0.4</td>
<td>0.48</td>
</tr>
</tbody>
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LV = left ventricular; RV = right ventricular.
affected, and that heart weights and myocardial water content after transplantation were either similar or reduced compared with control group hearts [17, 19, 23].

We used retrograde perfusion of donor hearts to avoid potential limitations of antegrade perfusion, particularly aortic valve incompetence. An additional benefit may be that this technique allows for flushing of any debris within the coronary arteries, a factor that is not usually an issue in the brain-dead donor, but may be of importance after the prolonged period of blood stasis these animals experienced. Clinically, right ventricular protection and consistency of cardioplegia distribution have been cited as limitations with retrograde perfusion [24, 25]. We also noted reduced nutrient flow to the right ventricle in canine experiments that were designed to develop the perfusion parameters used in this study [24]. In the current study, central venous pressures in perfused animals were low, but we did use a more sophisticated inotropic agent strategy compared with previous 4-hour and 14-hour storage experiments [17, 23], which may be a reflection of greater right ventricular impairment. Interestingly, TUNEL data suggest that the right ventricle experienced a relatively greater reduction in cell death than the LV from retrograde perfusion compared with control group hearts (data not shown).

This study has several limitations. Many clinical DCDD donors experience a devastating neurologic injury that may impact cardiac function, a condition that was not replicated by our experiments. Our definition of cardiac or circulatory death and its associated unpredictability undoubtedly contributed to some of the variability noted in our data. On the other hand, we do think this led to more clinically relevant results. The majority of human recipients have some degree of pulmonary hypertension, whereas recipient animals in the current study presumably had normal pulmonary vascular resistances. Also, important differences in canine and human cardiac venous anatomy may have affected perfusate delivery to the myocardium. Based on our previous studies, right ventricular protection in particular actually appears worse in canines [24]. It is encouraging that despite this limitation, right ventricular function in retrograde perfused animals appeared preserved with moderate inotropic support after transplantation.

In conclusion, DCDD has the potential to increase the donor pool for heart transplantation. The definition of cardiac/circulatory death is an important factor affecting the warm ischemic time after withdrawal of life-sustaining treatment. Standardization of DCDD in line with societal guidelines can limit unnecessary injury to otherwise suitable donor organs. Retrograde machine perfusion appears promising for recovery of DCDD hearts, even using the most stringent definitions of cardiac or circulatory death. Further investigation of machine perfusion preservation in a human experimental model of DCDD appears warranted before clinical application of this technique.

Organ Transport Systems, Inc (Frisco, TX) provided the prototype perfusion device, LifeCradle, for this study.

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References


DISCUSSION

DR AFSHIN EHSAN (Providence, RI): Two questions. In the interest of clinical translation, how do these agonal times compare to what we see in human DCD (donation after cardiac death) cases?

Number two, what was the rationale behind using retrograde perfusion versus an antegrade perfusion model?

DR BRANT: The time lines for the agonal phase are actually very comparable, assuming that you’re talking about DCD donors that are either apneic or very close to apneic. In a recent 10-year review of DCD liver transplants, the authors showed that most of the donors, about 90% of them, progressed to death within the first 35 minutes, and then if you go out to 55 minutes, essentially all of them are captured.

As far as the retrograde perfusion technique, previous studies from our laboratory demonstrated that under certain circumstances aortic insufficiency can occur with antegrade perfusion, resulting in nonnutrient flow. We evaluated a retrograde model to avoid this potential issue.

DR EHSAN: So do you have any data in those animals that you did antegrade where the valve was competent, and if so, were there any differences between the two approaches in preservation?

DR BRANT: Yes, we do have data with competent aortic valves, and there are no functional differences when the aortic valve remains competent.

DR PAVAN ATLURI (Philadelphia, PA): You know, clinically oftentimes the part that we really struggle with, and the part that I’ve struggled with in some of these, especially when you’re talking about longer cold ischemic times, is in terms of RV (right ventricular) function. A lot of these patients have elevated pulmonary vascular resistances. You’ve got great data on preservation of LV (left ventricular) function. How did the right ventricle behave?

DR BRANT: We did not have any issues with right ventricular dysfunction separating from bypass in retrograde perfused hearts. There is concern about the preservation of the right ventricle with retrograde perfusion. We previously reported using microsphere studies that there is reduced perfusion to the right ventricle in canines, but this didn’t seem to translate into functional issues in this study.

As far as human hearts, we have not noticed significant differences in right and left ventricular perfusion with this technique. There are anatomical differences in cardiac venous drainage between dogs and humans, which may explain differences in these models.

DR ATLURI: In fact, I think you’re exactly right, because clinically we have shown quite a bit that the retrograde coronary sinus catheters are great at protecting the left ventricle, but depending on how far you advance it, the left ventricle can be compromised.

DR BRANT: Yes, that is definitely the case. We intentionally secure the cannula to the coronary sinus orifice only with a pursestring and do not inflate the balloon to avoid obstruction of cardiac veins draining the right ventricle.
DR SAVERIO LAFRANCESCA (Houston, TX): Very interesting data. I probably missed it, what kind of solution do you actually use for the perfusion?

DR BRANT: We use UWMPS, University of Wisconsin Machine Perfusion Solution, which is a solution that is currently used for machine perfusion of donor kidneys.

DR LAFRANCESCA: Okay. And you did not show a diagram of your system. So it’s an open system, so you pretty much keep on giving solution or you recirculate the same solution?

DR BRANT: It is a recirculating system with a cooler and oxygenator. The heart is attached to the inflow cannula within the preservation chamber. Effluent from the organ is then recirculated, maintaining a consistent temperature and flow rate.

DR LAFRANCESCA: It is an open system. So the fluid comes out and it goes back into the system by gravity or else?

DR BRANT: The preservation solution is recirculated by a roller pump.

DR LAFRANCESCA: Okay. Thank you.

DR MOHAMMED QUADER (Richmond, VA): Congratulations on your work. It’s wonderful to see your results. I have a couple of questions. How long were the hearts supported on the heart–lung machine before being weaned off after the transplantation? And, did you have to use any inotropic agents to support them?

DR BRANT: We had a standard reperfusion period of 1 hour for both groups after reimplantation before we attempted to separate from bypass. We did use inotropes and pressors—dobutamine 5 μg · kg⁻¹ · min⁻¹, vasopressin 2 U/h, and epinephrine 0.05 μg · kg⁻¹ · min⁻¹.

DR QUADER: Just a follow-up question. When you looked at the heart function posttransplantation with the PV (pressure-volume) loops, how were they compared to the baseline values?

DR BRANT: In the perfused group, PV loops were not different compared to baseline throughout the reperfusion period. In the static group, when data were obtainable, PRSW (preload recruitable stroke work) dropped below baseline values at several time points.

DR QUADER: Thank you. Congratulations again.

DR EHSAN: One other question. In the human situation we monitor the pressure in the coronary sinus in order to avoid injury. Did you look at pressures within the coronary sinus and did that in any way offer information as to the degree of lactate that was present or not?

DR BRANT: Pressure was monitored but no adjustments were made based on coronary sinus pressures. However, pressures generally were low. We did measure perfusate lactate levels and oxygen consumption. We found that there is initially a period of lactate washout and associated increased myocardial oxygen consumption. Levels increase for the first 30 minutes, decrease over the subsequent 30 minutes, and then stabilize for the remainder of the preservation interval. This is most likely related to lactate accumulation and oxygen debt from the warm ischemic interval in this DCD model.

DR ATLURI: I might have missed this. Do you have a set flow that’s a weight-based flow, or do you flow based on pressures and your metabolic by-products?

DR BRANT: It’s a set flow.