Perfusion Preservation versus Static Preservation for Cardiac Transplantation: Effects on Myocardial Function and Metabolism

David H. Rosenbaum, MD,[a] Matthias Peltz, MD,[a] J. Michael DiMaio, MD,[a] Dan M. Meyer, MD,[a] Michael A. Wait, MD,[a] Matthew E. Merritt, PhD,[b] W. Steves Ring, MD,[a] and Michael E. Jessen, MD[a]

Introduction: Continuous perfusion of donor hearts for transplantation has received increasing interest, but the effects on cellular metabolism, myocyte necrosis, and myocardial edema are not well defined.

Methods: Pig hearts were instrumented with sonomicrometry crystals and left ventricular catheters. Left ventricular function was quantified by the pre-load-recruitable stroke work (PRSW) relationship. Hearts were arrested with Celsior solution with 5.5mM ^{13}C-glucose added, and removed and stored in cold solution ($n = 4$) or placed in a device providing continuous perfusion of this solution at 10 ml/100 g/min ($n = 4$). After 4 hours of storage, left atrial samples were frozen, extracted, and analyzed by magnetic resonance spectroscopy. Hearts were then transplanted into recipient pigs and reperfused for 6 hours, with function measured hourly. At the end of the experiment, left ventricular water content and serum creatine kinase-MB isoenzyme levels were measured.

Results: Baseline left ventricular function was similar in both groups. During reperfusion, the volume-axis intercept of the PRSW relationship was significantly lower in hearts stored with continuous perfusion ($p < 0.05$), suggesting reduced contractile impairment. Magnetic resonance spectroscopy revealed a decrease in tissue lactate in hearts that received continuous perfusion. Serum creatine kinase-MB isoenzyme levels were higher hearts that had static storage (30.8 ± 9.0 vs 13.2 ± 2.7 ng/ml; $p < 0.05$). Left ventricular water content was similar in both groups (0.797 ± 0.012 vs 0.796 ± 0.014; $p = 0.45$).

Conclusions: Donor hearts sustain less functional impairment after storage with continuous perfusion. This technique reduces tissue lactate accumulation and myocardial necrosis without increasing myocardial edema and appears promising as a method to improve results of cardiac transplantation. J Heart Lung Transplant 2008;27:93–9. Copyright © 2008 by the International Society for Heart and Lung Transplantation.

Current methods of static preservation for heart transplantation limit safe storage intervals to less than 6 hours. Although outcomes are acceptable within this timeframe, registry data suggest an increase in the relative risk of recipient mortality at 1 year when graft ischemia is prolonged beyond 3 to 4 hours.[1] Although perfusion devices have been found beneficial in experimental and clinical studies in kidney transplantation, efforts to test perfusion preservation in cardiac transplantation have been confined to animal studies, with various levels of success.[2–9]

In theory, this preservation strategy should offer several advantages: it may allow ongoing aerobic metabolism for preservation of cellular energetics, maintenance of transmembrane ionic gradients, and support of reparative processes in ischemic myocardium. The flow of solution through the coronary bed may also wash out end products of anaerobic metabolism (such as lactate), thereby reducing intracellular acidosis. The relative importance of these potential effects is unknown, despite encouraging functional results in many studies.

A successful perfusion preservation strategy may enable surgeons to safely extend the donor ischemic time to allow improved donor–recipient matching or transplantation on an elective rather than urgent basis. Some experimental work has suggested that these benefits are possible, but initial clinical trials of perfusion preservation are likely to involve conventional storage intervals until the safety and efficacy are better defined. Our current understanding of myocardial me-

From the [a]Department of Cardiovascular and Thoracic Surgery, and the [b]Advanced Imaging Research Center, University of Texas Southwestern Medical Center, Dallas, Texas.

Submitted June 1, 2007; revised October 8, 2007; accepted October 9, 2007.

This study was supported by a grant from the Texas Advanced Technology Program (010019-0108-2003) and by a grant from Organ Transport Systems Inc, Frisco, Texas.

Reprint requests: Michael E. Jessen, MD, Department of Cardiovascular and Thoracic Surgery, University of Texas Southwestern Medical Center, 5323 Harry Hines Blvd, Dallas, TX 75390-8879. Telephone: 214- 645-7721; Fax: 214-645-9708. E-mail: michael.jessen@utsouthwestern.edu

 1053-2498/08/$–see front matter. doi:10.1016/j.healun.2007.10.006

tabolism during this form of donor graft protection is incomplete and will need to be advanced if the technique is to gain clinical acceptance. We therefore designed this study to test a continuous perfusion strategy for cardiac preservation in a large animal model for a clinically relevant storage interval using techniques that allow precise quantification of functional recovery and substrate metabolism.

MATERIALS AND METHODS

Experimental Protocol

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

Sixteen adult farm pigs weighing 45–60 kg were divided into 8 donor–recipient pairs and randomized to hypothermic static preservation or hypothermic perfusion preservation with a perfusion device (LifeCradle, Organ Transport Systems Inc, Frisco, TX). Donor hearts were stored for 4 hours, implanted into recipient animals, and reperfused for 6 hours. Celsior organ preservation solution (Sangstat Medical Corp, Fremont, CA), supplemented with 1-g/L (5.5mM) U-^{13}C-labeled glucose to provide a substrate for evaluation of cellular metabolism, was used for storage of all hearts.

Anesthetic Protocol

Each animal was premedicated with 0.07-mg/kg intramuscular atropine and 4.4-mg/kg Telazol (Fort Dodge Animal Health, Overland Park, KS). The animal was intubated and ventilated with 100% oxygen at V_T of 10 ml/kg, rate of 10/min, and positive end expiratory pressure of 5 cm H_2O. Anesthesia was maintained with 1% to 4% isoflurane. Central venous pressure, arterial pressure, and an electrocardiogram were continuously monitored. Ventilator settings were adjusted based on arterial blood gas measurements to keep the P_{CO_2} at 35 to 45 mm Hg, pH 7.35 to 7.45, and oxygen saturation above 95%.

Donor Protocol

After sternotomy and exposure of the heart, animals were instrumented and baseline myocardial function was measured. Animals received intravenous heparin (300 U/kg). The aorta was clamped, the heart was arrested with 1 liter of modified Celsior solution through an ascending aortic cannula, and the donor cardiectomy was completed.

Hearts randomized to the Static Preservation Group were stored in a container filled with 1 liter of modified Celsior and placed in an ice chest. Hearts randomized to the Perfusion Preservation Group were attached to the perfusion device by a connector in the ascending aorta that provided a continuous flow of oxygenated, modified Celsior solution at a flow rate of 10 ml/100 g of heart tissue/min at 5° ± 2°C. This device uses an electrically powered roller pump that delivers a continuous, non-pulsatile flow of the solution into the ascending aorta. The preservation solution (2 liters total capacity) is oxygenated by an in-line membrane oxygenator, and all of the solution is recycled throughout the storage interval. In the Perfusion Preservation Group, a small polyethylene catheter was placed in the coronary sinus for serial measurements of oxygen tension.

Recipient Protocol

After induction of anesthesia, the recipient animal was placed on cardiopulmonary bypass (CPB) and the heart was excised to coincide with the end of the donor heart storage interval. The preserved heart was implanted into the recipient animal using the bicaval orthotopic transplant technique. During implantation, topical cooling was applied to the hearts in both groups with the infusion of cold Ringer's lactate, as has been described clinically.[10] Animals received 1 g of methylprednisolone before the aorta was unclamped. After the aortic clamp was removed, hearts were defibrillated with 5 to 20 J, if necessary. If 6 attempts at defibrillation were unsuccessful, 40 mEq of potassium chloride was administered into the CPB circuit. This caused a brief period of asystole, followed by restoration of sinus rhythm. An intravenous infusion of dobutamine was started at 5 μg/kg/min.

The animal was weaned from CPB after 1 hour of reperfusion. The left ventricular (LV) micromanometer catheter was reinserted, and the previously placed sonomicrometry crystals were connected to the preamplifier. Pressure-volume data were collected in triplicate over a range of filling conditions produced by draining blood into the cardiotomy reservoir (emptying curves) at 2, 3, 4, 5, and 6 hours post-reperfusion. After 6 hours of reperfusion, arterial blood was collected for determination of creatine kinase-MB isoenzyme (CKMB) levels and the animal was euthanized. An LV tissue sample was collected to measure water content.

Measurement of Ventricular Performance

Left ventricular function was quantified by a load-independent method that has been previously described in a porcine transplant model.[11] After exposure of the donor heart, 4 sonomicrometry crystals (Sonometrics Corp, London, Ontario, Canada) were attached to the subendocardium in the minor and major axes of the LV. These remained in situ during storage and reperfusion. A micromanometer-tipped catheter (Millar,

Houston TX) was inserted into the LV cavity through the apex. The catheter was removed before harvest and reinserted after implantation. Pressure and dimension data were collected at a rate of 250 Hz and digitized. Data were acquired, stored, and analyzed using SonoLab and CardioSOFT software (Sonometrics Corp, London Canada).

Pressure-volume loops were derived over the cardiac cycle before explantation (baseline) and after 2, 3, 4, 5, and 6 hours of reperfusion. At each time point, a series of pressure-volume loops was created over a range of filling conditions. Stroke volume was calculated from the integral (area) of each pressure-volume loop and plotted against the end-diastolic volume of each loop. The slope of this regression is termed pre-load-recruitable stroke work (PRSW), and is considered a load-independent index of contractility.[12] The volume axis intercept of the PRSW relationship (V_0) was also derived from these data at each time point. Changes in V_0 have been shown to represent a better discriminator of post-preservation contractile dysfunction than the slope of the PRSW relationship in a porcine heart transplant model.[11]

Assessment of Substrate Metabolism

Samples of left atrial appendage were harvested at the end of preservation and immediately freeze-clamped in liquid nitrogen. The tissue was stored at −80°C in a freezer and later extracted with perchloric acid. Purified extracts were reconstituted in deuterium oxide and pH was adjusted to 7.0 to 7.4 for magnetic resonance spectroscopy (MRS). ^{1}H MRS with and without ^{13}C decoupling were acquired with a 14.1-Tesla Varian spectrometer (Varian Inc, Palo Alto, CA) operating at 600 MHz over a spectral width of 8000 Hz for evaluation of metabolism of exogenous glucose by evaluating ^{13}C incorporation into metabolic intermediates.[13] Lactate concentrations were quantified with ^{1}H MRS by comparison to an internal standard.

Measurement of Myocardial Water Content

A sample of LV tissue was collected, blotted free of blood, weighed, and placed in an oven for desiccation. The sample was weighed daily. When a constant weight was reached on consecutive measurements, water content was calculated as (wet weight − dry weight)/wet weight.

Statistical Analysis

Results are reported as mean and standard error of the mean. Groups were compared with a 2-sided *t*-test using SigmaStat statistical software (Systat Software Inc, San Jose, CA). When outcome variables were measured at multiple time points, a repeated-measures analysis of variance was applied. Significance was set at $p < 0.05$.

RESULTS

Cardiac Function

Cardiac function was assessed from pressure-volume data acquired over a period of gradual reduction in LV filling and expressed as (1) the slope of the PRSW relationship and (2) the V_0 of the PRSW relationship. Left ventricular function was similar at baseline in both groups of animals. During the reperfusion interval, hearts stored with continuous perfusion demonstrated higher values of the slope of the PRSW relationship, although the difference was not statistically significant (Figure 1A). However, V_0 was significantly lower in hearts stored with perfusion preservation during the reperfusion interval (Figure 1B). Seven hearts in this study developed ventricular fibrillation that required administration of potassium chloride to regain sinus rhythm. One heart in the Perfusion Preservation Group regained sinus rhythm spontaneously after it was unclamped.

Cardiac Metabolism

Hearts in the Perfusion Preservation Group extracted dissolved oxygen continuously throughout the 4-hour storage interval (Table 1). Analysis of left atrial tissue extracts by ^{1}H MRS revealed less than 1% glutamate enrichment in both groups of animals. As the natural abundance of ^{13}C is 1.1%, no oxidation of exogenous labeled glucose was detected.

Very little intracellular lactate accumulated during organ preservation in the perfused hearts. This was readily apparent from inspection of spectra of each group and comparison of the lactate peaks to those from intracellular alanine (Figure 2). When absolute tissue lactate concentrations were calculated by comparison with an internal standard, samples collected at the end of the preservation interval from static stored hearts had a 14-fold higher lactate level than those stored with perfusion preservation (3.10 ± 0.69 μmol/g wet weight vs 0.22 ± 0.03 μmol/g wet weight; $p < 0.05$; Figure 3).

Myocardial Necrosis and Edema

Myocardial necrosis was estimated from blood levels of CKMB drawn at 6 hours post-reperfusion. The CKMB levels were significantly higher in recipient animals that obtained hearts stored with static preservation compared with levels in animals that received continuous perfusion organs (30.8 ± 9.0 ng/ml vs 13.2 ± 2.7 ng/ml; Figure 4, $p = 0.04$). Myocardial edema was not significantly different between groups at 6 hours post-reperfusion (0.797 ± 0.012 vs 0.796 ± 0.014; Table 2, $p = 0.45$).

DISCUSSION

Perfusion preservation of donor hearts has been a research interest of transplant surgeons; yet, clinical

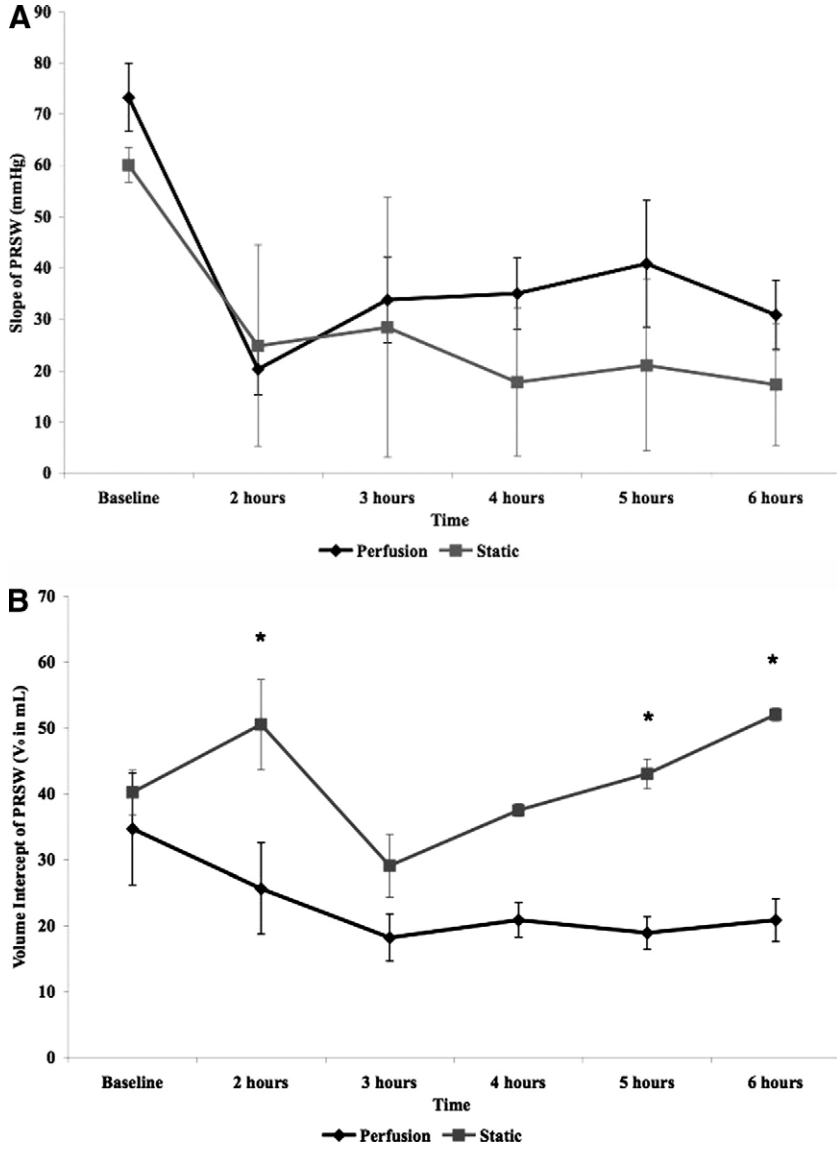

Figure 1. (A) Slope of the pre-load-recruitable stroke work (PRSW) relationship during the course of the experiment. No significant difference between perfused hearts and statically stored hearts was observed ($p = 0.26$). (B) Volume axis intercept (V_0) of the PRSW relationship during the course of the experiment. The overall trend of V_0 was significantly lower in the perfused hearts compared with the statically stored organs ($p < 0.01$). Data are presented with the standard error of the mean; *$p < 0.05$ at specific time points.

adoption of this technique has been hampered by the perceived complexity and expense of perfusion systems and by a general satisfaction with current techniques. However, the International Society of Heart and Lung Transplantation registry data demonstrate that 1-year mortality in heart transplant recipients approximately doubles in cases where donor ischemic time reaches 6 hours compared with results observed with donor ischemic times of 3 hours or less, and primary graft failure remains responsible for 40% of deaths within the first month after transplantation.[1] Improved preservation techniques, there-

Table 1. Variables Measured During Perfusion Preservation

Storage time	Temp (°C)	Inflow PO_2 (mm Hg)	Coronary sinus PO_2 (mm Hg)	Δ PO_2 (mm Hg)	Lactate (mmol/Liter)
1 hour	7.1 ± 1.1	1111 ± 44	526 ± 132	585 ± 88	0.03 ± 0.06
2 hours	6.3 ± 1.0	1061 ± 62	639 ± 184	422 ± 135	0.23 ± 0.17
3 hours	6.4 ± 1.9	1062 ± 28	565 ± 20	477 ± 77	0.13 ± 0.15
4 hours	5.3 ± 0.7	1136 ± 50	446 ± 67	690 ± 73	0.20 ± 0.18

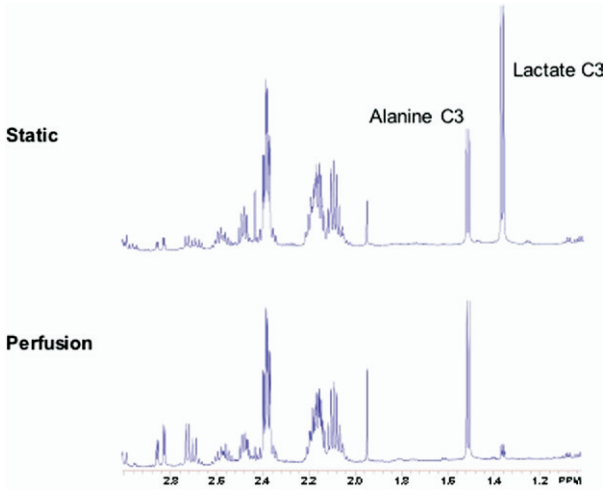

Figure 2. Representative magnetic resonance spectra from extracts of left atrial tissue at the end of storage. Methyl group protons of lactate and alanine C3 carbons are identified. Lactate (in relation to alanine) is greatly reduced in the perfused hearts.

fore, could lead to substantial improvements in the results of transplantation.

A growing body of information in other organ systems (particularly kidneys) suggests that providing a continuous supply of oxygenated perfusion solution to stored organs leads to improved outcomes after transplantation. The theoretic basis of these improvements is that the continuous delivery of oxygen and substrates maintains cellular adenosine triphosphate content by allowing ongoing aerobic metabolism. However, perfusion preservation may also remove end products of metabolism that could accumulate during storage (leading to intracellular acidosis) or generate oxygen free radicals during reperfusion.

The results of any myocardial protective strategy depend on the duration of the storage interval and on the storage environment provided to the organ. In this

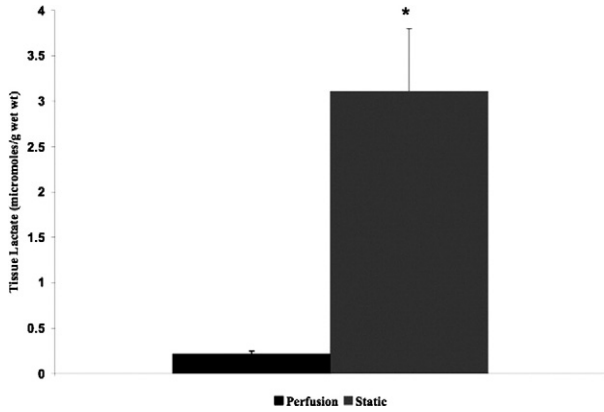

Figure 3. Tissue lactate concentrations in left atrial tissue collected at the end of the storage interval. Data are presented with the standard error of the mean; $*p < 0.05$.

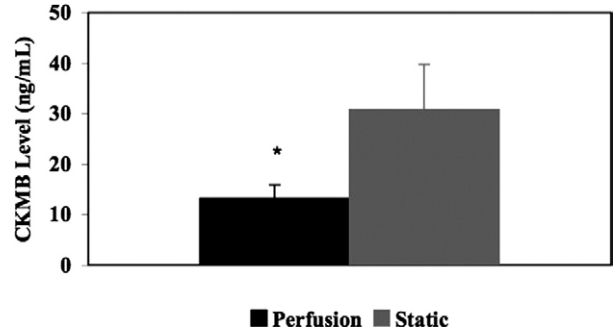

Figure 4. Creatine kinase-MB isoenzyme (CKMB) levels measured in arterial blood after 4 hours of storage and 6 hours of reperfusion. Data are presented with the standard error of the mean; $*p < 0.05$.

study, we selected a common clinical storage interval because initial clinical trials of perfusion preservation are likely to involve conventional donor ischemic times. We chose Celsior solution because prior studies of perfusion preservation in stored rat hearts demonstrated that metabolism of exogenous glucose and oxygen consumption were higher in hearts stored with this solution compared with an intracellular solution.[6] The ^{13}C-enriched glucose was provided in the solution to enable quantification of incorporation of label into citric acid cycle intermediates, as previously described.[13]

Hearts stored with perfusion preservation demonstrated improved functional recovery compared with static stored hearts, with significantly lower V_0 values measured throughout reperfusion. The PRSW slope values were not significantly different between groups, but Ryan et al[11] have demonstrated that the volume axis intercept of the PRSW relationship (V_0), not the slope, is a better discriminator of the severity of dysfunction.[11] This functional superiority is in agreement with other large animal studies that used a variety of devices, solutions, temperatures, and storage intervals.[2–9] Of note, we observed a modest functional improvement despite a relatively brief storage interval, suggesting that benefits may be derived from this technique even within timeframes that are typically considered "safe" with conventional protection strategies.

The degree of functional impairment observed after transplantation was substantial, particularly in the Static Preservation Group, and appeared greater than would be expected from clinical experience. Our selection of a porcine model may have played a role. In a porcine transplant study, Rao et al[3] found that an ischemic time

Table 2. Myocardial Water Content After Storage and Reperfusion

Group	Water content
Static	0.797 ± 0.021
Perfusion	0.795 ± 0.020

of 4 hours resulted in severe myocardial injury, with only 3 of 8 transplanted pig hearts successfully weaned from CPB. Other investigators have reported similar difficulties in establishing stable hemodynamics after preservation of porcine hearts under a variety of conditions.[14]

Hearts stored in the preservation device were found to extract dissolved oxygen throughout the storage period. This implies that oxidative metabolism was maintained with perfusion preservation. A surprising finding was that MRS done on left atrial samples at the end of the preservation interval did not demonstrate any significant incorporation of ^{13}C into citric acid cycle intermediates. This is in contrast with observations made in rat hearts preserved with an identical solution and studied with the same MRS method.[6] Several factors may explain these conflicting results. First, oxygen consumption without ^{13}C incorporation could be explained by oxidation of endogenous energy sources such as glycogen or triglycerides. This experiment used a low flow rate, low perfusion pressure, and cold temperature, which may affect the metabolism of exogenous nutrient sources. In addition, insulin was not provided in this preservation solution, and glucose uptake may have been impaired. The MRS analysis was conducted on left atrial tissue rather than on ventricular myocardium where a greater metabolic rate may be expected. Finally, a species effect may play a role.

Magnetic resonance spectra demonstrated a dramatic decrease in tissue lactate accumulation in atrial tissue at the end of the storage interval in the Perfusion Preservation Group compared with the Static Preservation Group. This may result from complete oxidation of glucose through pyruvate dehydrogenase and the citric acid cycle, without production of lactate. Alternatively, continuous perfusion may washout lactate formed from anaerobic metabolism. Small amounts of lactate measured in the perfusion fluid during 4 hours of storage suggest that washout may have had at least a partial role. Of note, all tissue lactate observed was unlabeled, suggesting that glycolysis of glucose from the preservation solution was not a contributor. Regardless of whether reduced lactate accumulation is a result of ongoing aerobic metabolism or continuous washout, lower lactate levels appear to correlate with improved post-reperfusion graft function. Neely and Grotyohann[15] have demonstrated that accumulation of anaerobic glycolytic products (lactate, hydrogen ion, or reduced nicotinamide adenine dinucleotide) may result in irreversible damage to the myocardium. Rao et al[16] demonstrated that post-reperfusion lactate release was an independent predictor of myocardial dysfunction. The increased CKMB levels measured in animals receiving static stored hearts in the current study is also consistent with greater ischemic damage that occurs with simple cold preservation.

One concern with the use of a perfusion device is the potential for the development of myocardial edema. Several different perfusion devices are under development by a number of manufacturers, and these devices differ in the method of perfusate delivery (pulsatile vs continuous vs beating heart) and perfusion solution composition. Poston et al[9] demonstrated that continuous perfusion led to edema and impaired diastolic function after 24 hours of storage compared with static preservation. Similarly, Nickless et al[2] demonstrated that after 5 hours of storage and 30 minutes of reperfusion, perfused hearts had 7% higher water content compared with their static counterparts.[2] In contrast, Hassanein et al[7] demonstrated a greater degree of edema in statically stored hearts after 12 hours of preservation and 2 hours of reperfusion compared with perfused organs. We found no significant difference in myocardial water content after 4 hours of preservation and 6 hours of reperfusion between hearts undergoing static storage and perfusion preservation. The perfusion conditions may be important determinants of myocardial edema development, but it is encouraging that our protocol results in significant metabolic enhancements without an associated increase in tissue edema. This technology may offer an opportunity to improve early results after cardiac transplantation.

REFERENCES

1. Taylor DO, Edwards LB, Boucek MM, et al. Registry of the International Society of Heart and Lung Transplantation: Twenty-third Official Adult Heart Transplantation Report–2006. J Heart Lung Transplant 2006;25:869–79.
2. Nickless DK, Rabinov M, Richards SM, Conyers RA, Rosenfeldt FL. Continuous perfusion improves preservation of donor rat hearts: importance of the implantation phase. Ann Thorac Surg 1998;65: 1265–72.
3. Rao V, Feindel CM, Weisel RD, Boylen P, Cohen G. Donor blood perfusion improves myocardial recovery after heart transplantation. J Heart Lung Transplant 1997;16:667–73.
4. Oshima K, Morishita Y, Yamagishi T, et al. Long-term heart preservation using a new portable hypothermic perfusion apparatus. J Heart Lung Transplant 1999;18:852–61.
5. Tsutsumi H, Oshima K, Mohara J, et al. Cardiac Transplantation Following a 24-H Preservation Using a Perfusion Apparatus. J Surg Res 1996;96:260–7.
6. Peltz M, He T, Adams GA, et al. Perfusion preservation maintains myocardial ATP levels and reduces apoptosis in an ex vivo rat heart transplantation model. Surgery 2005;138:795–805.
7. Hassanein WH, Zellos L, Tyrrell TA, et al. Continuous perfusion of donor hearts in the beating state extends preservation time and improves recovery of function. J Thorac Cardiovasc Surg 1998; 116:821–30.
8. Rao V, Feindel CM, Cohen G, Borger MA, Boylen P, Ross HJ. Is profound hypothermia required for storage of cardiac allografts? J Thorac Cardiovasc Surg 2001;122:501–7.
9. Poston RS, Gu J, Prastein D, Gage F, et al. Optimizing donor heart outcome after prolonged storage with endothelial function anal-

ysis and continuous perfusion. Ann Thorac Surg 2004;78: 1362–70.
10. Jessen ME, Meyer DM, Moncrief CM, Wait MA, Melamed NB, Ring WS. Reducing neurologic complications after cardiac transplantation: technical considerations. J Cardiac Surg 1993; 8:546–53.
11. Ryan JB, Hicks M, Cropper JR, et al. The preload recruitable stroke work relationship as a measure of left ventricular conctractile dysfunction in porcine cardiac allografts. Eur J Cardiothorac Surg 2002;22:738–45.
12. Glower DD, Spratt JA, Snow ND, et al. Linearity of the Frank-Starling relationship in the intact heart: the concept of preload recruitable stroke work. Circulation 1985;71:994–1009.
13. Jones JG, Hansen J, Sherry AD, Malloy CR, Victor RG. Determination of acetyl-CoA enrichment in rat heart and skeletal muscle by ^{1}H nuclear magnetic resonance analysis of glutamate in tissue extracts. Anal Biochem 1997;249:201–6.
14. Ferrara R, Marcsek P, Guidollet J, Berthet C, Dureau G. Lack of successful reanimation of pig hearts harvested more than ten minutes after death. J Heart Lung Transplant 1995;14:322–8.
15. Neely JR, Grotyohann LW. Role of glycolytic products in damage to ischemic myocardium. Circ Res 1984;55:816–24.
16. Rao V, Ivanov J, Weisel RD, Cohen G, Borger MA, Mickle DAG. Lactate release during reperfusion predicts low cardiac output syndrome after coronary bypass surgery. Ann Thorac Surg 2001; 71:1925–30.